Acta Biologica Hungarica 58 (2), pp. 225–233 (2007)
DOI: 10.1556/ABiol.58.2007.2.9

ULTRASTRUCTURE OF ENDOTHELIUM IN OVULES OF *PENSTEMON GENTIANOIDES* POIR. (SCROPHULARIACEAE) AT MATURE EMBRYO SAC PHASE

FERUZAN DANE, GÖKSEL OLGUN and NURAN EKİCİ*

Biology Department, Science and Art Faculty, Trakya University, 22030 Edirne, Turkey

(Received: July 27, 2006; accepted: October 5, 2006)

In this study ultrastructural differences between endothelial cells of different location in *Penstemon gentianoides* have been examined with electron microscope at mature embryo sac phase. Embryo sac is of the *Polygonum* type and surrounded by endothelium except the micropylar region. The cuticle is located primarily around the chalazal three-fourths of the embryo sac. Endothelium cells around the chalaza and toward the micropylar region are rich in cytoplasmic organelles. The cytoplasm of endothelial cells near the central cell has large vacuoles and few organelles. There are also plasmodesmas on the anticlinal walls of endothelial cells. The endothelium and the micropylar integumentary cells play a role in transport of metabolites into the embryo sac.

Keywords: Penstemon gentianoides – megagametophyte – endothelium – ultrastructure

INTRODUCTION

The inner epidermis of the inner integument of bitegmic ovules and that of the single integument of unitegmic ovules, in many plant species, becomes differentiated as a specialized layer of radially stretched cells with dense cytoplasm and a large nucleus [13]. The origin of these cells can be also nucellar [24]. The presence of endothelium is recorded in 65 families of dicotyledons and 7 families of monocotyledons [4]. According to Swamy & Krishnamurthy [30], a typical endothelium occurs only in dicotyledons. It constitutes an important diagnostic feature in 47% of them. The general correlation between endothelium and the unitegmic-tenuinucellate condition is well known, but its occurrence is seen in many bitegmic and crassinucellate families as well as *Papilionaceae* [2] and *Paeoniaceae* [6]. Endothelial coverage of the

* Corresponding author; e-mail: nuranekici@yahoo.com

embryo sac varies not only in diverse families but also in different species of the genus. The life span of endothelium is extremely variable. In some taxa it is ephemeral, whereas in others its activity begins during post-fertilization stages and it persists in the mature seed. Endothelium provides a range of variations with regards to its distribution, morphology, cytology, differentiation and behaviour [13]. It appears to fulfil diverse functions at different stages of development. It has high concentration of proteins, RNA, carbohydrates, ascorbic acid and enzymes [17]. It is believed that endothelium helps in coordinating growth in the ovule, canalizes nutrition to the embryo sac and later performs the protective function. And also provides nourishment to the developing embryo and endosperm [2].

Although endothelium provides an interesting object for study of ovular morphogenesis, there are few investigations conducted at the ultrastructural level, so that any generalization is difficult to make. Ultrastructural studies on the integuments in embryo stage have been made on *Pisum sativum* [14], *Medicago sativa* [27], *Phaseolus vulgaris* [34], *Melilotus alba* [26], *Glycine max* [5, 9] and *Vicia faba* [11]. In mature embryo sac stage these cells have been investigated in *Helianthus annuus*

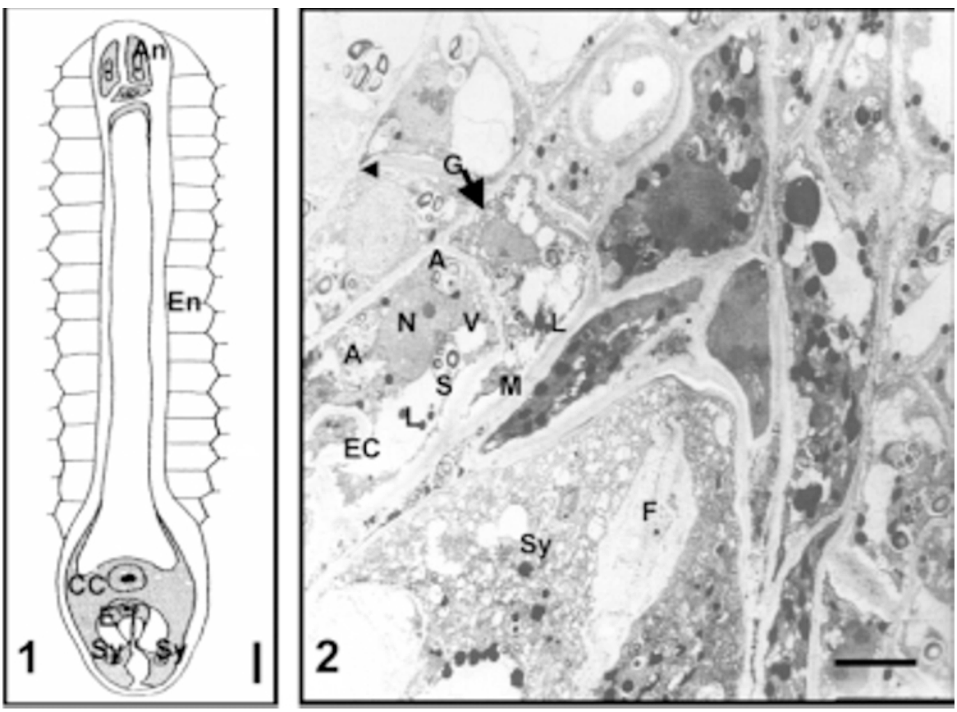

Fig. 1. Longitudinal view of mature embryo sac of *Penstemon gentianoides* with endothelial cells. Antipodals (An), central cell (CC), egg (E), endothelium (En), synergid (SY). Bar: 10 µm – *Fig. 2.* Endothelial cells toward the micropylar pole of mature embryo sac of *Penstemon gentianoides*. Amyloplast (A), endothelial cell (EC), filiform apparatus (F), Golgi bodies (G) (arrow), lipid bodies (L), mitochondria (M), nucleus (N), starch (S), synergid (Sy), vacuole (V). Bar: 3 µm

[18], in *Jasione montana* [3, 7], in *Linum usitatissimum* [33] and in *Calendula officinalis* [21]. In *Crepis tectorum* [10] the endothelium has been examined at uni- and four-nucleate stages of embryo sac.

The embryological investigations of some members of *Scrophulariaceae* family were made with light microscopy [1, 12, 19, 22, 23, 31]. Ultrastructural studies with male and female gametophytes of *Scrophulariaceae* family are very seldom and it was the megagametophyte of *Torenia fournieri* has firstly been studied from this family [32]. Ultrastructure of *Penstemon gentianoides* Poir.'s mature megagametophyte has also been studied [20] but up to now nothing has been published on the ultrastructure of the endothelium of *Scrophulariaceae* members to our best knowledge.

The purpose of this study was to give some information on the ultrastructure of the endothelium in mature embryo sac stages of *P. gentianoides* to shed more light on the general organization of its embryo sac as well as establishing its possible similarities to the embryo sac of other angiosperms.

MATERIAL AND METHODS

Penstemon gentianoides Poir. *(Scrophulariaceae)* flowers were collected from the Botanical Garden of the University of California at Berkeley and all the preparations were done at the same university. Ovules (from flower buds in 14–17 mm length) were fixed in 4% glutaraldehyde (GA) in 0.1M cacodylate buffer pH 6.8, for 2 hours. The ovules were then washed several times in buffer, fixed overnight with 2% buffered OsO_4 and dehydration was made with gradually increasing aceton-propylenoxide series with staining in 70% acetone containing 1% uranyl nitrate overnight. The material was embedded in Spurr's medium [29]. 0.5–1 μm thick semi-thin sections were stained with methylene blue (1%) using 0.02 M NaOH to control pH and observed using light microscopy. Thin sections were cut on a Porter-Blum ultramicrotome with a glass or diamond knife. Sections were stained on grids with lead citrate [25] for one minute and observed with a Zeiss EM 9A electron microscope.

RESULTS

Penstemon gentianoides has anatropous and unitegmic ovules. The female gametophyte was 8-nucleated monosporic *Polygonum* type and it is surrounded by endothelium before fertilization. The mature embryo sac has a narrow, elongated chalazal portion and an enlarged, oval micropylar area. It consists of egg apparatus (2 synergids and egg), central cell and 3 antipodals. Egg apparatus was located at the micropylar side of the embryo sac (Fig. 1). Synergids have well developed filiform apparatus (Fig. 2) and the highly vacuolate central cell in which the fusion nucleus is located near the egg. In the chalazal side there are 3 antipodal cells and they degenerate in advanced stages of development (Fig. 3). The inner epidermis of the integu-

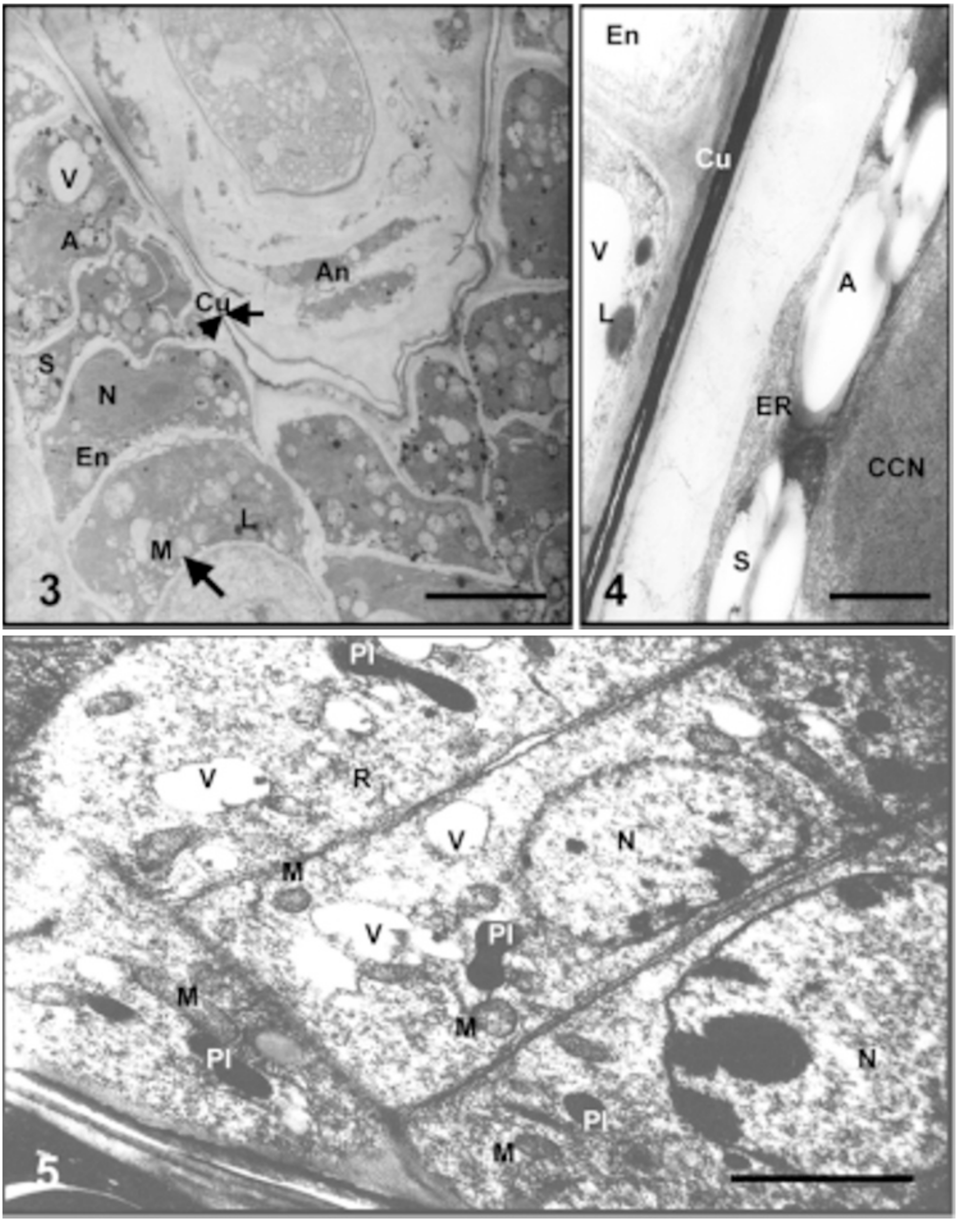

Fig. 3. Endothelial cells at the chalazal pole of mature embryo sac of *Penstemon gentianoides*. Amyloplast (A), antipodals (An), cutin (Cu) (arrows), endothelium (En), lipid bodies (L), mitochondria (M), nucleus (N), starch (S), vacuole (V). Bar: 5 µm – *Fig. 4.* Cutin accumulation in the cell wall of endothelial cell neighboring to central cell in *Penstemon gentianoides*. Amyloplast (A), central cell nucleus (CCN), cutin (Cu), endothelium (En), endoplasmic reticulum (ER), lipid bodies (L), starch (S), vacuole (V). Bar: 0.5 µm – *Fig. 5.* Endothelial cells near the chalaza before fertilization in *Penstemon gentianoides*. Nucleus (N), mitochondria (M), plastid (Pl), vacuole (V). Bar: 3 µm

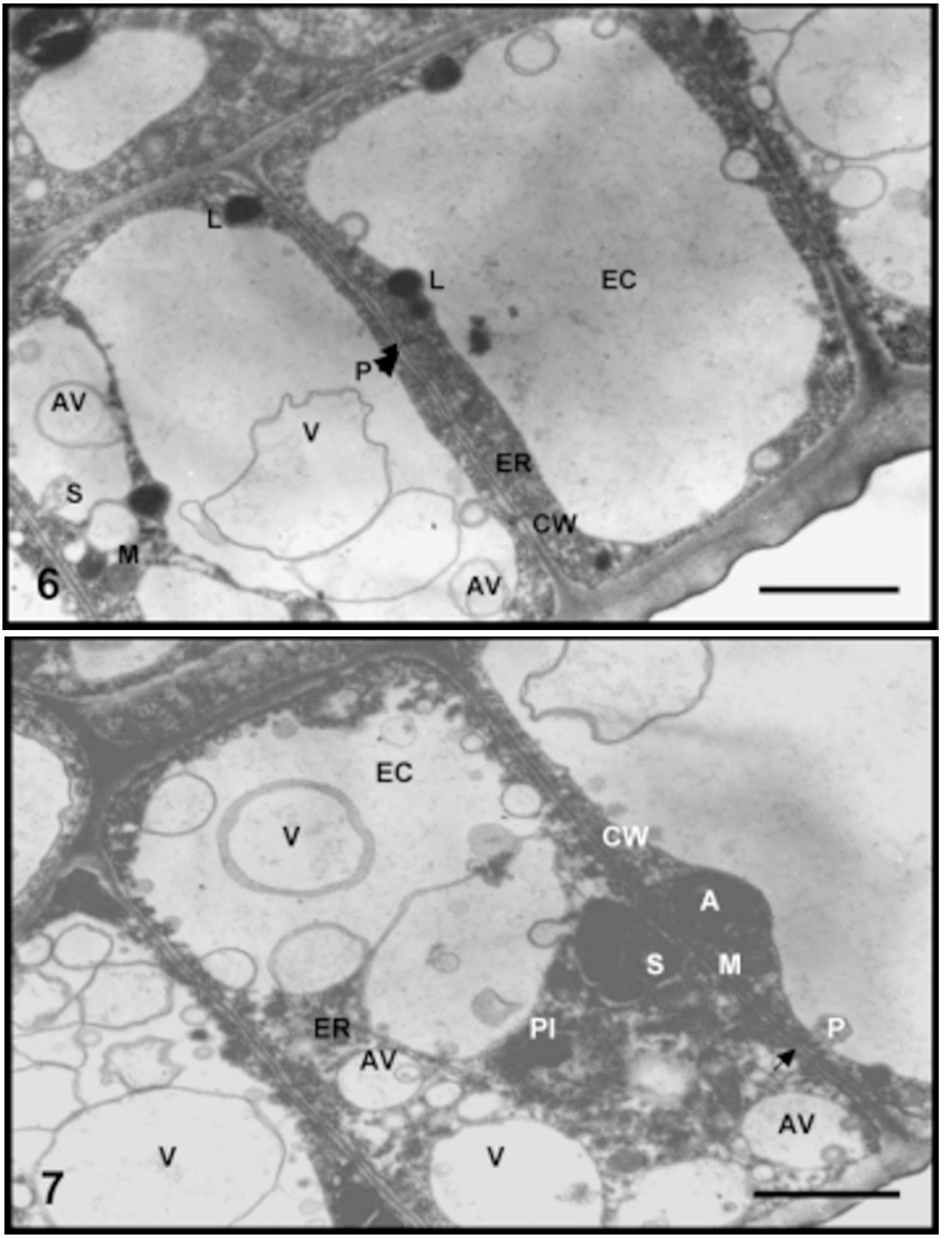

Fig. 6. Endothelial cells near the central cell in mature embryo sac phase of *Penstemon gentianoides*. Bar: 3 µm. Autophagic vacuole (AV), cell wall (CW), endothelial cell (EC), endoplasmic reticulum (ER), lipid bodies (L), mitochondria (M), plasmodesma (P), starch (S), vacuole (V) – *Fig. 7.* Endothelial cells near the central cell with vacuoles in mature embryo sac phase of *Penstemon gentianoides*. Bar: 3 µm. Amyloplast (A), autophagic vacuole (AV), cell wall (CW), endothelial cell (EC), endoplasmic reticulum (ER), mitochondria (M), plasmodesma (P), plastid (Pl), starch (S), vacuole (V)

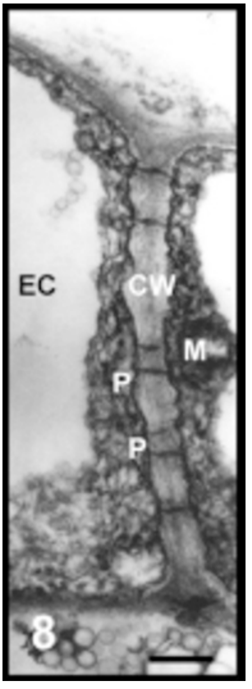

Fig. 8. Plasmodesmata in the cell wall between endothelial cells in mature embryo sac phase of *Penstemon gentianoides*. Bar: 1 µm. Cell wall (CW), endothelial cell (EC), mitochondria (M), plasmodesma (P)

ment is differentiated to form the integumentary tapetum or endothelium. The major part of the embryo sac lies in direct contact with the densely staining integument tapetum. The entire endothelium is also covered by cuticle. The cuticle is very thick along the narrow portion of the embryo sac (Fig. 4) but near the enlarged part, it becomes thinner and in some cases terminated (Fig. 2). A thin cuticle is also seen on the embryo sac wall (Fig. 3). The cutin layer belonging to endothelium cells and embryo sac unites near the central cell (Fig. 4).

When the female gametophyte is mature, there are numerous starch grains, particularly in the cells at the micropylar end of the integument. In the micropylar region, the endothelial cells were small and densely cytoplasmic with little vacuolation and have several organelles. Before fertilization, these cells are filled with numerous large amyloplasts. Nuclei and ER is seen. Mitochondria are dispersed throughout the cytoplasm. Golgi bodies formed are sporadic in number (Fig. 2).

The endothelial cells near the chalazal region were rich in mitochondria and lipid bodies (Fig. 3). They showed ultrastructural characteristics of meristematic cells. These cells have dense cytoplasm with little vacuoles, poorly developed ER, abundant free ribosomes, mitochondria, small number of plastids and dictyosomes (Fig. 5). Subsequently, they elongate and develop thick walls on the side adjacent to the embryo sac and probably cuticular in nature (Fig. 4). Some of the endothelial cells near to a central cell have dense and less vacuolated cytoplasm, nevertheless some of them were highly vacuolated (Figs 6, 7). Big vacuoles contain small vacuoles and cytoplasmic material. They seem to be autophagic vacuoles. In these cells, a few amyloplasts, mitochondria, lipid bodies and the rough ER is extensive and oriented parallel to the long axis of the cell (Figs 6, 7). The anticlinal wall of the endothelial cells is smooth shaped and includes many plasmodesma (Fig. 8).

DISCUSSION

In this study in *P. gentianoides,* a very conspicuous endothelium has been observed all along the side of the central cell and chalazal region, a feature that is common to other members of the *Scrophulariaceae* family [1, 19, 23].

According to this study the presence of numerous organelles in the endothelium of *P. gentianoides* indicates a metabolically active state of this tissue. Its ultrastructural aspects are similar to that of *Linum usitatissimum,* [33], *Helianthus annuus* [18], *Jasione montana* [3, 7], in *Calendula officinalis* [21] and *Saintpaulia ionantha* [17]. The endothelium of *P. gentianoides* has ultrastructural features since they have mitochondria, autophagic vacuoles, and plasmodesmas. Because of this they may be involved in “translocation of materials to the embryo sac”. The assignment of a secretory and nutritional role to the endothelium has been shown in other species of angiosperms [2, 13, 17, 28].

The cutinized walls of endothelium prevent the movement of digestive enzymes into nucellar cells or embryo sac [17]. The thickness, uniformity and continuity of the cuticular layer are variable not only in diverse species but also at different place in the same species. In *Digitalis*, *Scrophularia* and *Scoparia* the cuticle layer is either absent or thin near the micropylar region [1], in *Torenia* [32] it is thin at the submicropylar region, and in *Linaria* it is absent at the poles [23]. The thickness of the cuticular layer alters in relation to the major developmental change in the ovule. In *Saintpaulia ionantha* [17] the cuticle becomes very thin in some place; and often, the cuticle has small pores in it that were not seen in *P. gentianoides.*

The cuticle layer is at different place in various taxa. This indicates that there is no single pathway for transfer of nutrients from endothelium to embryo sac. Erdelska [7] reported that the cuticle disappeared at the level of the egg apparatus for *Jasione montana* just before fertilization, and she refered to this area as a transfer region. The presence of the transfer region; for example, in *Digitalis*, *Scrophularia* and *Scoparia* [1], in *Helianthus annuus* [18], *Proboscidea louisianica* [16], *Saintpaulia ionantha* [17] is at the micropylar region, in *Torenia* [32], in *Jasione montana* [3, 7] that is at the submicropylar region near the central cell, *Quercus gambelii* [15] and *Bellis perennis* [8] at the chalazal part.

In conclusion, according to observations in this study and the findings in the literature, it is started that the endothelium of *P. gentianoides* is similar to that in some species of *Scrophulariaceae.* The transfer region for nutrients is at the micropylar region but the thickness of cuticle layer surrounding *P. gentianoides'* embryo sac shows some differences from other members of this family.

REFERENCES

1. Arekal, G. D. (1963) Embryological studies in Canadian representatives of the tribe *Rhinantheae, Scrophulariaceae*. *Can. J. Botany 41,* 267–303.
2. Bakar, N., Algan, G. (1997) Electron microscopic examination of the development of the endothelium in *Trifolium pratense* L. during embryogenesis. *Turkish J. Botany 21,* 137–144.
3. Berger, C., Olga, E. (1973) Ultrastructural aspects of the embryo sac of *Jasione montana* L. cell walls. *Caryologia 25,* 109–120.
4. Bouman, F. (1984) The ovule. In: Johri, B. M. (ed.) *Embryology of Angiosperms.* Berlin, Springer-Verlag, pp. 123–157.
5. Chamberlin, M. A., Horner, H. T., Palmer, R. G. (1993) Nutrition of ovule embryo sac and young embryo in soybean: an anatomical and autoradiographic study. *Can. J. Botany 71,* 1153–1168.
6. Dane, F., Olgun, G. (1998) *Paeonia tenuifolia* L. (*Paeoniaceae*)'da tohum taslağ°n°n yap° ve fonksiyonunun °ş°k ve elektron mikroskopu ile incelenmesi. *Marmara Universitesi Fen Bilimleri Dergisi 14,* 11–38.
7. Erdelska, O. (1975) Pre-fertilization development of ovule of *Jasione montana*. *Phytomorphology 25,* 76–80.
8. Engell, K., Petersen, G. B. (1977) Integumentary and endothelial cells of *Bellis perennis*. Morphology and histochemistry in relation to the developing embryo sac. *Botanisk Tidskrift 71,* 237–244.
9. Folsom, M. W., Cass, D. D. (1986) Change in the transfer cells distribution in the ovule of soybean after fertilization. *Can. J. Botany 64,* 965–972.
10. Godineau, J. C. (1971) Ultrastructure of the embryo sac of *Crepis tectorum* L. after cell formation and fusion of polar nuclei. *Ann. Univ. l'Assoc. Régionale Étude Rech. Sci.* pp. 78–88.
11. Johansson, M., Walles, B. (1994) Functional anatomy of the ovule in broad-bean (*Vicia faba* L.): ultrastructural seed development and nutrient pathways. *Ann. Botany 74,* 233–244.
12. Kapil, R. N., Masand, P. (1964) Embryology of *Hebenstreitia integrifolia* Linn. *Proc. Nat. Inst. Sci. India, 32B,* 218–232.
13. Kapil, R. N., Tiwari, S. C. (1978) The integumentary tapetum. *Botanical Rev. 44,* 457–490.
14. Marinos, N. G. (1970) Embryogenesis of the pea *(Pisum sativum).* The cytological environment of the developing embryo. *Protoplasma 70,* 261–279.
15. Mogensen, H. L. (1973) Some histochemical, ultrastructural, and nutritional aspects of the ovule of *Quercus gambelii*. *Am. J. Botany 60,* 48–54.
16. Mogensen, H. L. (1978) Synergids of *Proboscidea louisianica* (Martineaceae) before fertilization. *Phytomorphology 28,* 114–122.
17. Mogensen, H. L. (1981) Ultrastructural localization of adenosine triphosphatase in the ovules of *Saintpaulia ionantha* (Gesneriaceae) and its relation to synergid function and embryo sac nutrition. *Am. J. Botany 68,* 183–194.
18. Newcomb, W., Steeves, T. A. (1973) The development of the embryo sac of sunflower *Helianthus annuus* before fertilizations. *Can. J. Botany 51,* 863–878.
19. Olgun, G. (1979) Investigation on the comparative karyology and embryology (embryo sac and endosperm development) of *Digitalis viridiflora* Lindl. and *Digitalis lutea* L. *İstanbul Üniversitesi Fen Fakültesi Mecmuas°. Seri B44,* 1–22.

20. Olgun, G., Jensen, J. A. (1987) The ultrastructure of the egg apparatus and central cell of *Penstemon gentianoides* Poir. before fertilization (*Scrophulariaceae*). *İstanbul Üniversitesi Fen Fakültesi Mecmuas° Seri B52,* 35–45.
21. Plisko, M. A. (1971) An electron microscopic investigation of the characteristic features of megagametogenesis in *Calendula officinalis* L. *Botanicheskii Zhurnal* 56, 582–597. (In Russian.)
22. Raju, D. (1973) Embryological studies in the tribe *Selagineae agathelpis* Dubia. *Phytomorphology 23,* 194–205.
23. Raju, D., Arekal, G. D. (1978) Endosperm and embryo in *Linaria ramosissima*. *Phytomorphology 28,* 88–95.
24. Rau, M. A. (1951) The mechanism of nutrition in *Vigna catjang*. *New Phytologist 50,* 121–123.
25. Reynolds, E. S. (1963) The use of lead citrate of high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol. 17,* 208–212.
26. Roti-Michelozzi, G., Riggio-Bevilacqua, L., Boca, P. (1989) Development of the tissues surrounding the embryo in *Melilotus alba* Med. *Phytomorphology* 39, 157–160.
27. Sangduen, N., Kreitner, G. L., Sorensen, E. L. (1983) Light and electron microscopy of embryo development in perennial and annual *Medicago* species. *Can. J. Botany 61,* 837–849.
28. Seghal, C. B., Gifford, E. M. (1979) Developmental and histochemical studies of the ovules *Nicotiana rustica* L. *Botanical Gazette 140,* 180–188.
29. Spurr, A. R. (1969) A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res. 26,* 31–43.
30. Swamy, B. G., Krishnamurty, K. V. (1970) On the so-called endothelium in the monocotyledons. *Phytomorphology 20,* 262–269.
31. Taneja, S. (1970) Embryology, seed and fruit structure of *Hebenstreitia comosa*. Hochest, with systematic position of the family *Selagineacae*. *J. Ind. Botanical Soc. 48,* 300–310.
32. Van Der Pluijm, J. E. (1964) An electron microscope investigation of the filiform apparatus in the embryo sac of *Torenia fournieri* J. In: Linskens, H. F. (ed.) *Pollen Physiology and Fertilization.* North Holland Pub. Co. Amsterdam, pp. 8–16.
33. Vazart, B., Vazart, J. (1965) Infrastructure de I'ovule du lin, *Linum usitatissimum* L. I. L'assise jaquette ou endothelium. *Acad. Sci. Paris Compt. Rend. 261,* 2927–2930.
34. Yeung, E. C. (1983) Developmental changes in the branched parenchyma cells of bean-sead coat. *Protoplasma 118,* 225–229.