Protoplasma 116, 170–178 (1983)

PROTOPLASMA

Some Aspects of Organization and Histochemistry of the Embryo Sac of *Scilla sibirica* Sato

N. N. BHANDARI* and ANURADHA SACHDEVA

Department of Botany, University of Delhi

Received September 3, 1982
Accepted November 22, 1982

Summary

The present investigation deals with some of the organizational and histochemical aspects of the embryo sac of *Scilla sibirica*. Both the synergids and egg cell are invested by PAS-positive complete walls. The filiform apparatus comprises an elaborate system of fibrillar projections, showing extensive ramifications. The micropylar region of the embryo sac wall from where the filiform apparatus originates is composed of three distinct layers. On a histochemical basis it may be surmised that, unlike the egg cell, the synergids are metabolically very active. Two kinds of wall ingrowths (i) massive and highly branched very much akin to the filiform apparatus, and (ii) small tuberculate wall projections, are unique to the antipodal cells of *S. sibirica*. Small tuberculate projections have also been observed along the wall of the central cell adjacent to the nutrient-rich nucellar cells. The antipodals and the central cell show the presence of starch grains and abundant total proteins. All the cell types in the embryo sac of *S. sibirica* are structurally so organized as to meet the requirements of its nutrition during pre- and postfertilization development. The presence of abundant PAS-positive granular substance in the cells of nucellar epidermis probably establishes a gradient which assists in the pollen tube growth.

Keywords: Embryo sac; *Scilla sibirica;* Wall-ingrowths; Transfer cells.

1. Introduction

The female gametophyte in angiosperms exhibits tremendous variation in its ontogeny, organization and structure. In spite of exhaustive literature dealing with one or the other aspect of female gametophyte, a number of questions still remain disputable chiefly because of the availability of contrary data in different taxa. Although it is now clear that the synergids are highly active cells, all the functions they might be performing are not clearly understood. The nature of the filiform apparatus and presence or absence of the wall surrounding the synergids and the egg cell have long been an object of discussion. COCUCCI and JENSEN (1969) reported the occurrence of discrete walls around all the cells of the female gametophyte in *Epidendrum scutella*. These walls are, however, not continuous over a small area common to the central cell, the degenerating synergid and the egg cell. On the contrary, in many plants such as cotton (JENSEN 1965 a), *Crepis tectorum* (GODINEAU 1969), *Zea mays* (DIBOLL 1968), *Linum usitatissimum* (VAZART 1969), *Petunia* (VAN WENT 1970 a, 1970 b) and *Aquilegia* (VIJAYARAGHAVAN *et al.* 1972), the walls in the synergids and the egg cell thin down progressively from the pole to the central cell.

The mature embryo sac provides several instances where wall ingrowths have been reported in the synergids, in the form of filiform apparatus (see KAPIL and BHATNAGAR 1981), in the central cell in *Linum* (VAZART and VAZART 1966), *Capsella* (SCHULZ and JENSEN 1968 a, 1968 b), *Helianthus* (NEWCOMB and STEEVES 1971), *Aquilegia* (FOUGÈRE-RIFOT 1978), *Cortaderia* (PHILIPSON 1978) and *Paspalum* (YU and CHAO 1979), and in antipodal cells in *Zea mays* (DIBOLL and LARSON 1966), *Stipa elmeri* (MAZE and LIN 1975), *Eschscholtzia*, *Chelidonium* (FOUGÈRE-RIFOT 1979 a)

* Correspondence and Reprints: Department of Botany, University of Delhi, Delhi 110007, India.

0033-183X/83/0116/0170/$ 01.80

and *Paspalum longifolium* (YU and CHAO 1979). In such transfer cells the surface area of plasmalemma is increased which presumably facilitates greater exchange of nutrients (GUNNING 1977). The occurrence and chemical nature of wall ingrowths in correlation to the distribution of food reserves in and around the embryo sac would evidently furnish ample information concerning the translocation routes within the ovule and the various aspects of embryo sac nutrition.

The nucellus has an important storage function in the ovules. Histochemistry reveals predominantly the presence of starch and lipids in nucellar tissue (see TILTON and LERSTEN 1981). Besides some other metabolites, protein crystals (GORI 1976), tannins (UHL and MOORE 1971) ascorbic acid and many enzymes (MALIK and VERMANI 1975) have also been reported. It is speculated that nucellar stores are used up by the maturing embryo sac. The present histochemical study was undertaken to get a coherent account of organizational and functional aspects of the nucellus and different cells in the mature gametophyte.

2. Material and Methods

The ovaries of *Scilla sibirica* were collected at different developmental stages by one of us (NNB) during his stay at Geisenheim, West Germany in May 1979. The material was fixed in 10% aqueous solution of acrolein (E. Merck) at 0 °C for 24 hours. Dehydration, infiltration and embedding procedures outlined by FEDER and O'BRIEN (1968) were followed. The only modification made was in monomer mixture which comprised purified glycolmethacrylate (92.2 ml), 2,2'-Azobis (0.3 g) and polyethylene glycol 400 (7.5 ml). The sections were cut at 2.0 μm thickness with glass knives on a Spencer A 0 microtome with the help of a special adaptor for holding knives. Among histochemical moieties localized and also included here were the insoluble polysaccharides (PAS-reaction – FEDER and O'BRIEN 1968), total proteins (FISHER 1968 – modified) and DNA (FEDER and O'BRIEN 1968). Photomicrographs were taken on a Carl Zeiss (West Germany) Photomicroscope III.

3. Observations

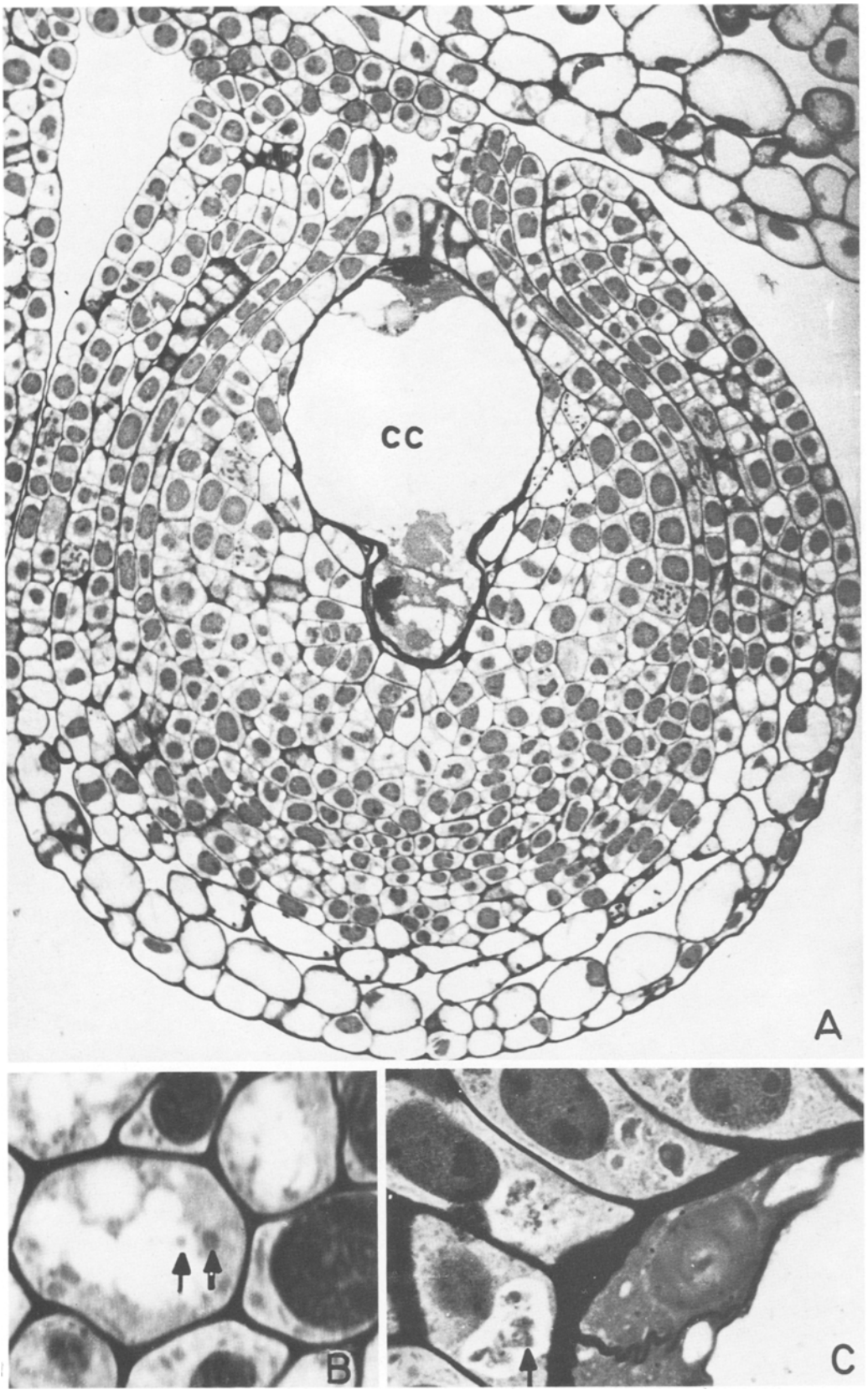
The mature embryo sac of *Scilla sibirica* Sato which develops in an anatropous, bitegmic and crassinucellate ovule, is a seven-celled structure prior to fertilization (Fig. 1 *A*). The shape of the embryo sac varies depending on the disposition of the antipodal cells in the chalazal region. When the three antipodal cells lie one above the other, the embryo sac appears oval – being broader in the micropylar region and gradually tapering down towards the chalaza. However, in other instances, the embryo sac becomes constricted towards the chalazal region to form a pouch in which three antipodal cells are lodged.

3.1. Synergids

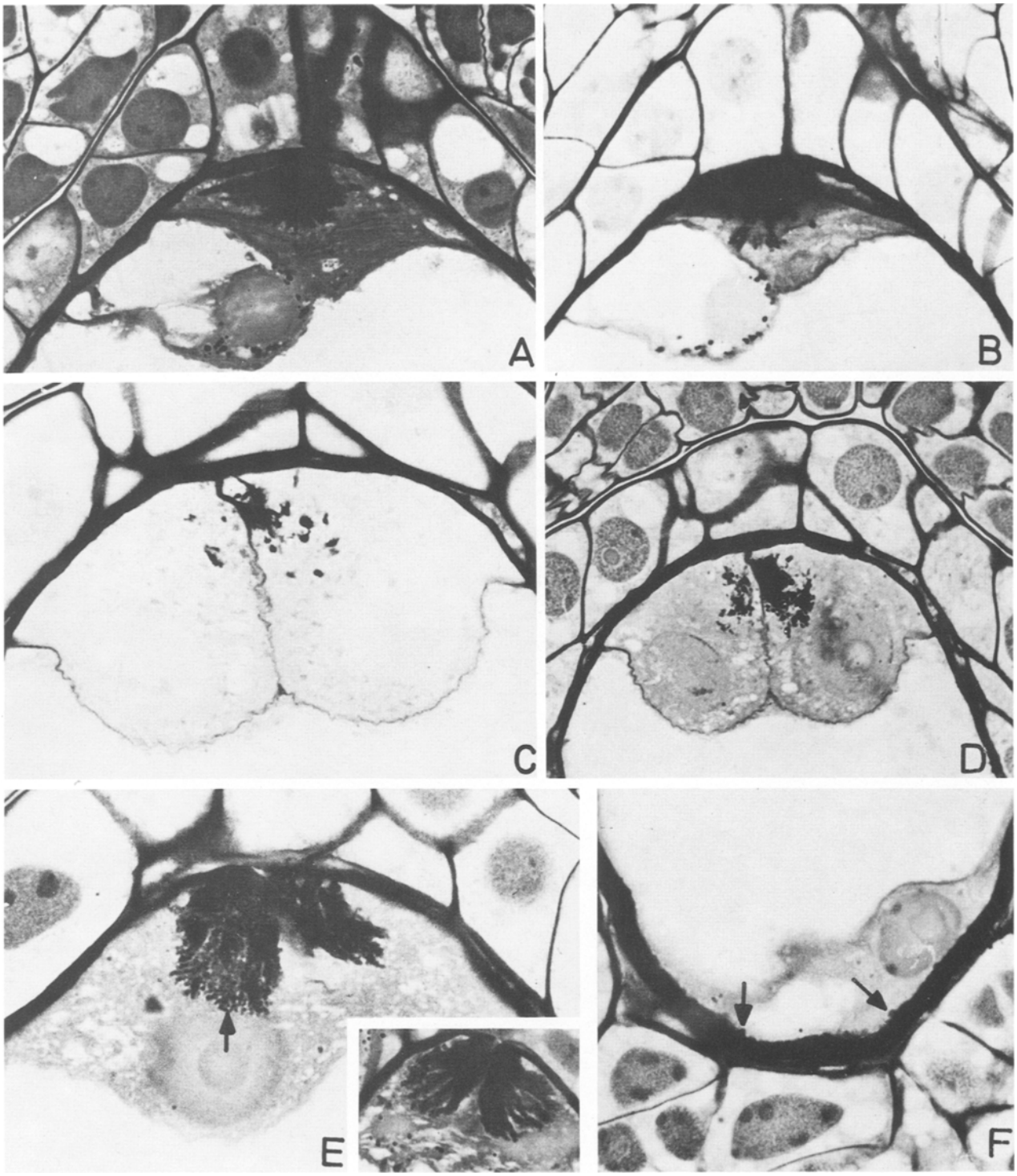
The two synergids lie juxtaposed to each other, containing a large, chalazally located, uninucleolate, spherical nucleus. The cytoplasm appears to be very rich in total proteins and has numerous small vacuoles. The nucleus and the nucleolus also stain densely for total proteins (Figs. 2 *D*, *E*). The filiform apparatus, which is the most conspicuous structure, in the synergid extends from the wall in the proximal portion of the cell upto almost its center (Fig. 2 *E*). It originates mainly from the wall along the micropylar region but finger-like extensions are also given out from upper part of the lateral wall separating the two synergids (Figs. 2 *C*, *D*). The site of origin of the filiform apparatus along the micropylar wall is composed of three distinct layers; an outer thin and PAS-positive, a middle lightly stained and amorphous, and an inner thick and strongly PAS-positive (Fig. 2 *E*). The filiform apparatus stains intensely for insoluble polysaccharides and comprises an elaborate coralloid system of fibrillar projections showing extensive ramifications (Fig. 2 *E*). Such projections in cross-section appear to have various shapes and dimensions. The synergids of *Scilla sibirica* are characterised by the presence of a complete wall all around the cells (Fig. 2 *C*). Intense staining following PAS-reaction reveals that its thickness is not uniform. The synergid wall at the micropylar end is thicker than that near the central cell. Numerous starch grains (PAS-positive, confirmed also by IKI staining) are observed in the synergid cytoplasm.

3.2. Egg

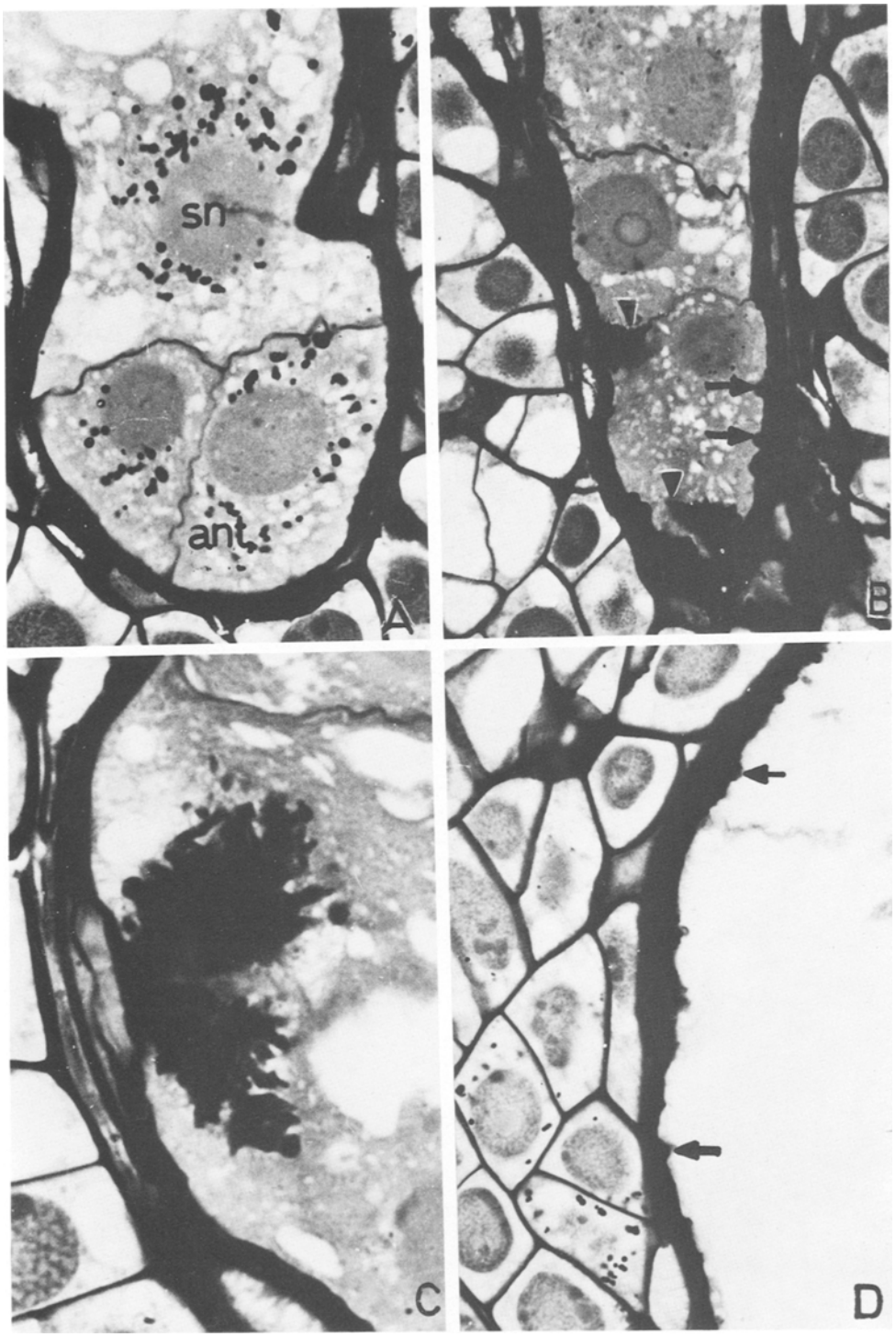
The egg cell is slightly larger than the synergid and measures 28 μm along its maximum length. The mature egg is centrally located vis-a-vis synergids which are juxtaposed. It is placed slightly below the synergids with respect ot the micropyle (Fig. 2 *A*). The egg cell, too, shows the presence of a complete wall around the cell (Fig. 2 *B*), which stains strongly for insoluble polysaccharides. Scanty cytoplasm is present near the cell periphery and around the nucleus. A major part of the proximal region of the egg cell is occupied by a single large vacuole (Figs. 2 *A*, *B*) and consequently the nucleus is pushed towards the chalazal end. The cytoplasm is rich in total proteins and also exhibits a few starch grains showing perinuclear arrangement (Figs. 2 *A*, *B*).



Figs. 1*A–C*. Ovule and nucellus, PAS-aniline blue staining. (*A*) Longisection of a bitegmic, crassinucellate ovule at the mature embryo sac stage. The embryo sac is constricted towards the chalazal region to form a pouch in which three antipodal cells are lodged. The secondary nucleus in central cell (*CC*) is present near the antipodal cells. × 550. (*B*) A portion of chalazal nucellar tissue. Note the presence of protein bodies (arrows) in the cytoplasm and vacuoles. The nuclei and nucleoli stain more intensely for total proteins than the cytoplasm. × 845. (*C*) A part of nucellar epidermis and embryo sac in longisection. The cells of nucellar epidermis are uniseriate and contain PAS-positive, granular material (arrow) in the vacuoles. × 845



Figs. 2*A-E*. Organised mature embryo sac; (*F*) Unorganised embryo sac, (*A*, *D-F*) PAS-aniline blue staining, (*B*, *C*) PAS-staining. (*A*) Longisection passing through micropylar region of an ovule showing egg cell and synergid. The egg cell shows a large proximal vacuole, scanty cytoplasm and a prominent nucleus with perinuclear starch grains. The synergid shows a prominent filiform apparatus. ×800. (*B*) Same, immediately next to (*A*), the egg cell shows a complete PAS-positive wall whereas the synergid depicts the presence of a PAS-positive matrix. ×800. (*C*) Longisection of an ovule showing two synergids lying juxtaposed and completely surrounded by pectocellulosic wall stained deeply for insoluble polysaccharides. The chalazal wall of the synergids and the lateral wall between the two synergids is undulating. The filiform apparatus extends from the lateral wall between the two synergids. ×1,220. (*D*) Same, section next to (*C*). The nucleus in each synergid lies in the distal half and numerous small vacuoles are interspersed in the dense cytoplasm which stains intensely for total proteins. ×667. (*E*) Longisection of an ovule passing through two synergids. The filiform apparatus (arrow) is an extension of the micropylar wall of the synergids and the lateral wall between the two synergids. The micropylar wall is composed of three distinct layers, viz., the outer thin layer, middle amorphous layer and the inner thick layer. The filiform apparatus is well developed and shows extensive ramifications. ×1,200. Inset (from another section) shows the filiform apparatus in the two synergids and appears like a mirror-image. ×1,200. (*F*) A chalazal portion of an unorganised embryo sac in longisection. Note the presence of small papillate wall ingrowths (arrows). ×800



Figs. 3*A–D*. The antipodals and central cell. (*A–D*) PAS-aniline blue staining. (*A*) Longisection of an ovule passing through the antipodal cells and a part of central cell. The secondary nucleus (*sn*) lies very close to the antipodal cells. The antipodal cells (*ant*) are densely cytoplasmic and show an intense reaction for total proteins. Many small vacuoles are interspersed in the cytoplasm of antipodal cells. Perinuclear starch grains are discernable both in the antipodal and the central cell. × 770. (*B*) Two distinct types of wall ingrowths are clearly visible in the antipodal cells. Tufts of wall ingrowths (arrow heads) are present in the chalazal tier in the vicinity of nucellar tissue and in the wall which is separating the two antipodal cells. The small papillate wall ingrowths (arrows) are present all along the wall of the antipodal cell adjoining the chalazal nucellar tissue. × 770. (*C*) A part of the antipodal cell in longisection. The region of the antipodal cell from which the tuft of wall ingrowths arise is seen to be divided into three zones. The tuft of wall ingrowths simulates the filiform apparatus and likewise shows extensive ramifications. × 2,250. (*D*) The wall of the central cell in the vicinity of nutrient-laden nucellar cells, shows small papillate wall projections (arrows). These projections are evenly distributed over the entire surface of the central cell wall. Note the presence of starch grains in the nucellar tissue, in the vicinity of central cell. × 920

3.3. Central Cell

The central cell has a large vacuole and a secondary nucleus which is present near the antipodal cells (Fig. 1 *A*). A peculiar feature in the central cell is the presence of numerous tuberculate ingrowths distributed more or less uniformly throughout the inner surface of its wall next to the nucellus (Fig. 3 *D*). These wall ingrowths are very small, singly located and measure up to 0.5 μm in length. Both the wall of the central cell and the wall ingrowths stain intensely and uniformly for insoluble polysaccharides. The scanty cytoplasm of the central cell amassed around the secondary nucleus stains deeply for total proteins. Numerous starch grains are also present in the vicinity of the secondary nucleus (Fig. 3 *A*).

3.4. Antipodal Cells

The three antipodal cells appear to be arranged in two tiers at the chalazal end of the embryo sac. The most remarkable feature of the antipodal cells is the presence of extensive wall ingrowths (Figs. 3 *B*, *C*) of two different kinds. Firstly, small tuberculate wall projections (Fig. 3 *B*) similar to those in the central cell (Fig. 3 *D*). These ingrowths are present on the chalazal and lateral walls of the antipodal cells. Secondly, very massive and highly branched wall projections (Fig. 3 *C*) which are akin to the filiform apparatus (Fig. 2 *E*).

These are present on the chalazal side of the lower antipodal tier and on either side of the wall separating the lower and the upper tiers. Such ingrowths are conspicuously absent on the wall separating the uppermost antipodal cell from the central cell. Both types of wall ingrowths are strongly PAS-positive and thus composed of pectocellulose. In the unorganised embryo sac, at the 8-nucleate stage, minute wall ingrowths in the chalazal region can be observed even when the three antipodal cells are not fully formed (Fig. 2 *F*).

A definite wall surrounds each of the three antipodal cells completely. It is thinner in the region where it is in contact with the central cell whereas on the lateral sides and in the chalazal region it is much thicker. The cytoplasm of the antipodal cells has many small vacuoles, and is moderately rich in total proteins. Large number of starch grains (Fig. 3 *A*) are also present. The antipodal nuclei show a more intense Feulgen reaction when compared to the other cells of the embryo sac, and are probably polyploid.

3.5. Nucellus

At the mature embryo sac stage there is a single persisting layer, the nucellar epidermis, in the proximal region of the embryo sac whereas on either side of the embryo sac the nucellus is multilayered. The chalazal nucellar tissue is rather massive. The epidermal cells of the nucellus are modified in the micropylar region to form a layer of very uniform rectangular cells. The nuclei of this layer are very rich in total proteins. Below the nucleus a prominent vacuole is present in each cell in which are present many groups of PAS-positive granular (-ve to IKI) material (Fig. 1 *C*). Such material is also found in numerous small vacuoles which are interspersed in the cytoplasm of these cells.

The cells of the nucellus on either side of the embryo sac are uninucleate and stain deeply for total proteins. Such cells in the vicinity of the embryo sac possess numerous starch grains. In the chalazal region the cells of nucellar tissue are more vacuolated as compared to the cells on either side of the embryo sac. The presence of numerous protein bodies staining strongly for total proteins (Fig. 1 *B*) has been observed. Each large protein body is surrounded by a vacuole while other smaller protein bodies are randomly distributed in the cytoplasm. They are spherical, smooth and comprise uniformly distributed proteinaceous material. At the mature embryo sac stage, cells of the chalazal nucellar tissue in the immediate vicinity of the embryo sac appear to be lacking in starch grains, although an increasing gradient of starch grains is observed in the cells towards the chalazal region.

4. Discussion

The study of mature gametophyte of *S. sibirica* reveals a high degree of structural differentiation among its four cell types thereby pointing to the complexity of its organization and function. The synergids of *S. sibirica* are highly polarized cells. The well developed filiform apparatus comprising a network of numerous finger-like projections stains strongly with PAS-reaction indicating their pectocellulosic composition as is also shown in the synergids of *Vanda* (ALVAREZ and SAGAWA 1965), *Zephyranthes rosea* and *Lagenaria vulgaris* (MALIK and VERMANI 1975), *Linum usitatissimum* (BHAT and VIJAYARAGHAVAN 1980), *Ranunculus sceleratus* (VIJAYARAGHAVAN and BHAT 1980) and *Argemone mexicana* (BHANDARI *et al.* 1980). In *Aquilegia vulgaris*, however, FOUGÈRE-RIFOT (1975) found the occurrence of PAS-negative filiform

apparatus. Proteins have been described in the filiform apparatus of *Gossypium* (JENSEN 1965 a), *Paspalum* (CHAO 1971), *Aquilegia* (VIJAYARAGHAVAN *et al.* 1972), *Proboscidea* (MOGENSEN 1978), *Eschscholtzia californica* (FOUGÈRE-RIFOT 1979 a), *Agave parryi* (TILTON and MOGENSEN 1979) and *Ornithogalum caudatum* (TILTON 1981). TILTON (1981) also reported the presence of RNA in the filiform apparatus of *O. caudatum* in addition to carbohydrates and proteins. The filiform apparatus, the so called wall-membrane apparatus of the synergid cells in *S. sibirica* (present work) and other spp. so far investigated is characteristic of transfer cells. It amplifies the surface of plasma membrane thereby helping in short distance transfer of solutes (GUNNING and PATE 1969, 1974, PATE and GUNNING 1972). The filiform apparatus has also been implicated in the secretion of chemotropic substances which are thought to guide the growth of the pollen tube into the synergid. In both *Agave parryi* (TILTON and MOGENSEN 1979) and *Ornithogalum caudatum* (TILTON 1981) the proximal end of the nucellar cap cells has numerous vesicles which appear to be fusing with or pinching off from the plasmalemma. It is speculated that these vesicles transport materials from the synergids into the nucellar cap. In *Paspalum longifolium* and *P. orbiculare* (CHAO 1971, 1977) the micropyle is filled with a PAS-positive substance of integumentary and nucellar origin and this substance is also believed to influence the final stages of pollen tube growth. In *S. sibirica* at the mature embryo sac stage, the nucellar epidermal cells capping the egg apparatus and in the vicinity of the synergids, show the presence of abundant granular PAS-positive substance included in the vacuoles. The walls of some of the cells of the nucellar epidermis in contact with the embryo sac also show the formation of wall ingrowths which extend into their cytoplasm. It is possible that the PAS-positive granular substance in the vacuoles of nucellar epidermis is synthesized under the influence of the synergids. In *S. sibirica* this substance, along with the filiform apparatus, might set up a gradient of chemotactic material which may influence the final phases of pollen tube growth.

The presence of a PAS-positive wall surrounding entirely the synergid of *S. sibirica* is another feature worthy of mention. This is contrary to the majority of earlier observations where the wall around the chalazal part of the synergid common to themselves and the central cell is absent (see JENSEN 1974). COCUCCI and JENSEN (1969) have described the synergid wall being continuous over the entire cell in *Epidendrum scutella*. In *Capsella bursa-pastoris* (SCHULZ and JENSEN 1968 a) also a wall is present all around the synergid, but it presents a honeycomb appearance at the chalazal region. The synergids of *Ornithogalum caudatum*, are also unusual in being invested completely by a cell wall which appears to be uniform and without discontinuities. Obviously there must exist in *S. sibirica* a system of hydrolytic enzymes in the pollen tube, in the synergids themselves or the central cell, to bring about the release of sperms entrapped in the degenerating synergid to effect double fertilization.

The cytoplasm of the synergids of *S. sibirica* appear to be highly active metabolically as revealed by their intense staining for total proteins and presence of numerous starch grains. Similar intense staining for total proteins has also been observed in the synergids of cotton (JENSEN 1965 a), *Vanda* (ALVEREZ and SAGAWA 1965), *Capsella bursa-pastoris* (SCHULZ and JENSEN 1968 a), *Nicotiana rustica* (SEHGAL and GIFFORD 1979), *Ranunculus sceleratus* (VIJAYARAGHAVAN and BHAT 1980), and *Ornithogalum caudatum* (TILTON 1981). The synergids appear to be very specialized secretory cells as evidenced by the presence of a well developed filiform apparatus and large deposits of proteins and starch.

The egg cell of *S. sibirica* has a complete wall surrounding it as also occurs in *Plumbago capensis* (CASS 1972) and *Ornithogalum caudatum* (TILTON 1981). In *Epidendrum scutella* the wall extends over the chalazal portion of the egg but is interrupted or honeycombed (COCUCCI and JENSEN 1969). However, in the majority of the plants investigated the cell wall of the egg in the distal region is lacking (see JENSEN 1974, KAPIL and BHATNAGAR 1981). In those plants where a definite wall is present its dissolution must be brought about before the entry of the sperm. Such precise studies are desirable.

The presence of starch grains and a moderate amount of total proteins indicate that the egg is not a metabolically inactive cell. The egg cells of *Helianthus annuus* (NEWCOMB 1973) and *Plumbago zeylanica* (CASS and KARAS 1974) also appear to be active metabolically. However, the egg cell does not show an abundance of organelles and appears to be quiescent in comparison to the synergids. This is supported by histochemical features in *Stellaria media* (PRITCHARD 1964), cotton (JENSEN 1965 b), *Capsella bursa-pastoris* (SCHULZ and JENSEN 1968 b), *Zea mays* (DIBOLL 1968), *Petunia* (VAN WENT 1970 b), *Stipa elmeri* (MAZE and LIN 1975) and *Nicotiana tabacum* (MOGENSEN and SUTHAR 1979).

The central cell has a PAS-positive wall which shows small projections distributed all along the wall in contact with the nutrient-rich nucellar cells. Such

projections have also been reported in *Linum usitatissimum* (VAZART and VAZART 1966), *Helianthus annuus* (NEWCOMB and STEEVES 1971), *Hibiscus* hybrids (ASHLEY 1975), *Triticum aestivum* (MORRISON *et al.* 1978), *Haemanthus katherinae* (NEWCOMB 1978), *Aquilegia vulgaris* (FOUGÈRE-RIFOT 1978), *Cortaderia jubata* (PHILIPSON 1978), *Paspalum* (YU and CHAO 1979) and *Chelidonium majus* and *Eschscholtzia californica* (FOUGÈRE-RIFOT 1979 b). The central cell in *S. sibirica* appears to be more metabolically active as compared to the egg as revealed by the presence of wall ingrowths, intense reaction for proteins and presence of starch grains. This high rate of metabolism of the central cell is no doubt related to its nutritional requirements for the preparation of this cell to initiate the development of endosperm.

The antipodal cells in *S. sibirica* have a pectocellulosic wall all around their surface. Two types of wall ingrowths viz., in the form of a tuft of branched finger-like projections and smaller individual protuberances arise from the walls. It is important to note that although the amplification of wall projections is achieved during maturation of the female gametophyte, their initiation can already be observed along the chalazal part of the unorganized eight-nucleate embryo sac. Such wall extensions are normally lined by plasmalemma (GUNNING and PATE 1974). The occurrence of wall ingrowths in the cells of *S. sibirica* obviously amplify the surface area for the absorption of metabolites in these cells and is indicative of their role as transfer cells (GUNNING and PATE 1974). This is further supported by the observation that they lie in the vicinity of chalazal nucellar tissue which is rich in proteins and starch. The frequency of wall ingrowths in different antipodal cells indicate that the cells in the chalazal tier absorb nutrients more efficiently, as compared to the antipodal cell in the upper tier. The presence of wall proliferations between the cells of two tiers indicate that the nutrients might be translocated from one tier to the other. Light microscopic observations on the antipodal wall ingrowths have been made only in a few spp., such as *Paspalum* (YU and CHAO 1979), *Cortaderia jubata* (PHILIPSON 1978), *Papaver somniferum* (BHANDARI and BHARGAVA 1982) and *Papaver nudicaule* (OLSON and CASS 1981), though ultrastructural studies have been made on the wall proliferation of a number of antipodal cells (see KAPIL and BHATNAGAR 1981).

The antipodal nuclei may be polyploid and the cytoplasm includes abundant total proteins and starch grains. The location of antipodal cells in the proximity of nutrient-rich nucellar cells, their histochemical reactions, and the presence of wall proliferations suggest that they play an important role in the nutrition of the embryo sac in *S. sibirica*.

It may be emphasized that although wall projections have been reported in one or the other cells of the mature embryo sac, *S. sibirica* to our knowledge is the only example where not only all the cells of the female gametophyte, *i.e.*, synergids, central cell and antipodals show wall projections but also the nucellar epidermal cells show the PAS-positive granules and rare occurrence of wall projections. In fact all cell types in the embryo sac of *S. sibirica* are structurally so organized as to meet the requirements of its nutrition during maturation and postfertilization changes.

Acknowledgements

We are grateful to Professor R. N. KAPIL for facilities, to Dr. S. P. BHATNAGAR for allowing us to use Photomicroscope III, Carl Zeiss (West Germany). One of us (A.S.) gratefully acknowledges the financial assistance from the Centre of Advanced Study in Botany in the form of Junior Research Fellowship.

References

ALVAREZ, M. R., SAGAWA, Y., 1965: A histochemical study of embryo sac development in *Vanda* (*Orchidaceae*). Caryologia **18**, 241–249.

ASHLEY, T., 1975: Zygote shrinkage and subsequent development in some *Hibiscus* hybrids. Planta **108**, 303–317.

BHANDARI, N. N., SOMAN, P., BHARGAVA, M., 1980: Histochemical studies on the female gametophyte of *Argemone mexicana* L. Cytologia **45**, 281–291.

– BHARGAVA, M., 1982: Wall ingrowths in the antipodal cells in *Papaver somniferum* L. Beitr. Biol. Pflan. (in press).

BHAT, U. (nèe DHAR), VIJAYARAGHAVAN, M. R., 1980: Distribution of insoluble polysaccharides in *Linum usitatissimum* – zygote to seedling. Caryologia **45**, 65–75.

CASS, D. D., 1972: Occurrence and development of filiform apparatus in the egg of *Plumbago capensis*. Amer. J. Bot. **59**, 279–283.

– KARAS, I., 1974: Ultrastructural organization of the egg of *Plumbago zeylanica*. Protoplasma **81**, 49–62.

CHAO, C. Y., 1971: A periodic acid Schiff's substance related to the directional growth of pollen tube into embryo sac in *Paspalum* ovules. Amer. J. Bot. **58**, 649–654.

– 1977: Further cytological studies of a periodic acid Schiff's substance in the walls of *Paspalum orbiculare* and *P. longifolium*. Amer. J. Bot. **64**, 921–930.

COCUCCI, A. E., JENSEN, W. A., 1969: Orchid embryology: The megagametophyte of *Epidendrum scutella*. Kurtziana **5**, 23–38.

DIBOLL, A. G., 1968: Fine structural development of the megagametophyte of *Zea mays* following fertilization. Amer. J. Bot. **55**, 797–806.

– LARSON, D. A., 1966: An electron microscopic study of the mature megagametophyte of *Zea mays*. Amer. J. Bot. **53**, 391–402.

FEDER, N., O'BRIEN, T. P., 1968: Plant microtechnique: Some principles and new methods. Amer. J. Bot. **55**, 123–142.

FISHER, D. B., 1968: Protein staining of ribboned epon sections for light microscopy. Histochemie **16**, 92–96.

FOUGÈRE-RIFOT, M., 1975: L'édification de l'appareil filiforme et l'évolution cytoplasmique des synergides du sac embryonnaire d'*Aquilegia vulgaris*. C. R. Acad. Sci., Paris **280**, 2445–2447.

– 1978: La cellule centrale du sac embryonnaire d'*Aquilegia vulgaris* L. des noyaux polaires aux noyaux d'albumen Laiteux. Soc. bot. Fr., Actualités botaniques no. **1-2**, 207–213.

– 1979a: Les synergides du sac embryonnaire d'*Eschscholtzia californica* (Papavéracée): étude ultrastructurale et cytochimique de l'appareil filiforme. Rev. Cytol. Biol. végét. Bot. **2**, 199–211.

– 1979b: Les cellules de transfert dans le sac embryonnaire de quelques phanérogames. 104° Congrès national des sociétés savantes, Bordeaux. Sciences fasc. II, 105–116.

GODINEAU, J. C., 1969: Ultrastructure des synergides chez quelques Compocées. Rev. Cytol. Biol. Vég. **32**, 209–226.

GORI, P., 1976: Protein bodies in the nucellus of *Euphorbia helioscopica*. J. Ultrastruct. Res. **54**, 53–58.

GUNNING, B. E. S., 1977: Transfer cells and their roles in transport of solutes in plants. Sci. Prog. Oxford **64**, 539–568.

– PATE, J. S., 1969: "Transfer cells"–plant cells with wall ingrowths, specialized in relation to short distance transport of solutes–their occurrence, structure and development. Protoplasma **68**, 107–133.

– – 1974: Transfer cells. In: Dynamic Aspects of Plant Ultrastructure (ROBARDS, A. W., ed.), pp. 441–480. London: McGraw-Hill Book Co.

JENSEN, W. A., 1965a: The ultrastructure and histochemistry of the synergids of cotton. Amer. J. Bot. **52**, 238–256.

– 1965b: The composition and ultrastructure of the nucellus in cotton. J. Ultrastruct. Res. **13**, 112–128.

– 1974: Reproduction in flowering plants. In: Dynamic Aspects of Plant Ultrastructure (ROBARDS, A. W., ed.), pp. 481–503. London: McGraw-Hill Book Co.

KAPIL, R. N., BHATNAGAR, A. K., 1981: Ultrastructure and biology of female gametophyte in flowering plants. International Rev. Cytol. **70**, 291–341.

MALIK, C. P., VERMANI, S., 1975: Physiology of sexual reproduction. I. A histochemical study of the embryo sac development in *Zephyranthes rosea* and *Lagenaria vulgaris*. Acta histochem. **53**, 244–280.

MAZE, J., LIN, S. C., 1975: A study of the mature gametophyte of *Stipa elmeri*. Canad. J. Bot. **53**, 2958–2977.

MOGENSEN, H. L., 1978: The synergids of *Proboscidia lousianica* before fertilization. Phytomorphology **28**, 114–122.

– SUTHAR, H. K., 1979: Ultrastructure of the egg apparatus of *Nicotiana tabacum* (*Solanaceae*) before and after fertilization. Bot. Gaz. **140**, 168–179.

MORRISON, I. N., O'BRIEN, T. P., KUO, J., 1978: Initial cellularization and differentiation of aleurone cells in the central region of the developing wheat grain. Planta **140**, 19–30.

NEWCOMB, W., 1973: The development of the embryo sac of sunflower *Helianthus annuus* before fertilization. Canad. J. Bot. **51**, 863–878.

– 1978: The development of cells in the coenocytic endosperm of African blood lily *Haemanthus katherinae*. Canad. J. Bot. **56**, 483–501.

– STEEVES, T. A., 1971: *Helianthus annuus* embryogenesis, embryo sac wall projection before and after fertilization. Bot. Gaz. **132**, 367–371.

OLSON, A. R., CASS, D. D., 1981: Changes in megagametophyte structure in *Papaver nudicaule* L. (*Papaveraceae*) following in vitro placental pollination. Amer. J. Bot. **68**, 1338–1341.

PATE, J. S., GUNNING, B. E. S., 1972: Transfer cells. Ann. Rev. Pl. Physiol. **23**, 173–196.

PHILIPSON, M. N., 1978: Apomixis in *Cortaderia jubata*. N. Z. J. Bot. **16**, 45–59.

PRITCHARD, H. N., 1964: A cytochemical study of embryo sac development in *Stellaria media*. Amer. J. Bot. **51**, 371–378.

SCHULZ, R., JENSEN, W. A., 1968a: *Capsella* embryogenesis: The synergids before and after fertilization. Amer. J. Bot. **55**, 541–552.

– – 1968b: *Capsella* embryogenesis: the egg, zygote and young embryo. Amer. J. Bot. **55**, 807–819.

SEHGAL, C. B., GIFFORD, E. M., JR, 1979: Development and histochemical studies of the ovules of *Nicotiana rustica* L. Bot. Gaz. **140**, 180–188.

TILTON, V. R., 1981: Ovule development in *Ornithogalum caudatum* (*Liliaceae*) with a review of selected papers on angiosperm reproduction. IV. Egg apparatus structure and function. New Phytol. **88**, 505–531.

– LERSTEN, N. R., 1981: Ovule development in *Ornithogalum caudatum* (*Liliaceae*) with a review of selected papers on angiosperm reproduction III. Nucellus and megagametophyte. New Phytol. **88**, 477–504.

– MOGENSEN, L. H., 1979: Ultrastructural aspects of the ovule of *Agave parryi* before fertilization. Phytomorphology **29**, 338–350.

UHL, N. W., MOORE, H. E., JR., 1971: The palm gynoecium. Amer. J. Bot. **58**, 945–992.

VAN WENT, J. L., 1970a: The ultrastructure of the egg and central cell of *Petunia*. Acta Bot. Néerl. **19**, 313–322.

– 1970b: The ultrastructure of the fertilized embryo sac of *Petunia*. Acta Bot. Néerl. **19**, 468–480.

VAZART, B., VAZART, J., 1966: Infrastructure du sac embryonnaire du Lin (*Linum usitatissimum* L.). Rev. Cytol. Biol. vég. **24**, 251–266.

VAZART, J., 1969: Organisation et ultrastructure du sac embryonnaire du Lin (*Linum usitatissimum* L.). Rev. Cytol. Biol. vég. **32**, 227–240.

VIJAYARAGHAVAN, M. R., BHAT, U., 1980: Synergids and antipodal cells in *Ranunculus sceleratus* Linn.–a histochemical approach. Proc. Indian natn. Sci. Acad. B **46**, 674–680.

– JENSEN, W. A., ASHTON, M. E., 1972: Synergids of *Aquilegia formosa*–their histochemistry and ultrastructure. Phytomorphology **22**, 143–159.

YU, S. H., CHAO, C. H., 1979: Histochemical studies of ovary tissues during the embryo sac development in *Paspalum longifolium* Roxb. Caryologia **32**, 147–160.