Phyton (Austria)	Vol. 23	Fasc. 1	31—38	15. 2. 1983

Embryo Sac Wall in *Iberis amara* and *Alyssum maritimum*

Histochemical and Some Fine Structural Aspects

By

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With 3 Plates (11 Figures)

Received September 1, 1981

Key words: *Iberis amara*, *Alyssum maritimum*; transfer cells, embryo sac wall, histochemistry, ultrastructure

Summary

Kumkum PRABHAKAR & M. R. VIJAYARAGHAVAN 1983. Embryo sac wall in *Iberis amara* and *Alyssum maritimum*, histochemical and some fine structural aspects. Phyton (Austria) 23 (1): 31—38. — English with German Summary.

The embryo sac wall, in both *Iberis amara* and *Alyssum maritimum*, stain well with PAS-reaction both at the micropylar and the chalazal ends After about heart-shaped stage of embryo, however, wall ingrowths in these two taxa are more pronounced at the chalazal end than at the micropylar end. In *I. amara* wall ingrowths at the chalazal end are prominent enough to be resolved under light microscope. The electron microscopic studies on *A. maritimum* reveal that at the micropylar end PAS-positive wall has labyrinth of ingrowths.

Zusammenfassung

Kumkum PRABHAKAR & M. R. VIJAYARAGHAVAN 1983. Die Wand des Embryosackes von *Iberis amara* und *Alyssum maritimum*, zur Histochemie und zum Feinbau. Phyton (Austria) 23 (1): 31—38, mit 11 Abbildungen auf 3 Tafeln. — Englisch mit deutscher Zusammenfassung.

Die Wand des Embryosackes von *Iberis amara* und *Alyssum maritimum* färbt sich mit der PAS- (= Perjodsäure-Schiff-)Reaktion deutlich am mikro-

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pylaren wie chalazalen Ende. Etwa nach dem herzförmigen Stadium des Embryos sind jedoch Zellwandeinstülpungen bei den beiden Species am chalazalen Ende deutlicher ausgeprägt als am Mikropylarpol. Bei *I. amara* sind die Zellwandeinstülpungen deutlich genug, um unter dem Lichtmikroskop erkannt zu werden. Die elektronenmikroskopischen Bilder von *A. maritimum* lassen erkennen, daß die PAS-positive Zellwand am mikropylaren Pol ein Labyrinth von Zellwandeinstülpungen enthält.

(Editor transl.)

1. Introduction

Plant cells with wall ingrowths are known to occur in a variety of situations (Gunning & Pate 1969, 1974). Occurrence of such projections in the cells/tissues involved directly (egg cell of *Plumbago*) or aiding in fertilization (synergids) or translocation (basal cell of embryo, antipodal cells of female gametophyte etc.) is well-known. Gunning & Pate (1969) have further suggested that selction pressures of physiological nature have shaped the formation of transfer cells.

The histological, histochemical and ultrastructural aspects of embryo sac wall, during different phases of embryogenesis, is poorly understood. The presence of wall ingrowths assigns it a role in absorption of nutrients from either nucellus or integuments. There are only a few reports on the modifications of embryo sac wall (Vazart & Vazart 1966, Marinos 1970, Newcomb & Steeves 1971, Schulz & Jensen 1974, Newcomb 1978, Yeung & Clutter 1978). Present study was undertaken to elucidate whether the entire surface of the embryo sac is absorptive or there are special sites, restricted on embryo sac wall, for absorption.

Accumulation of endosperm cytoplasm and nuclei at the chalazal end of the embryo sac is well-known (see Maheshwari 1950) and such accumulated portion of endosperm cytoplasm and nuclei is believed to act as a haustorium. Little is known regarding the histochemical nature of this portion of endosperm at the chalazal end and is postulated to play an important role in absorption due to presence of wall ingrowths (see also Schulz & Jensen 1974). Ontogenetical data of such wall modifications are not known and further it is also not clear whether this type of haustorium is active only during early, mid-, or late embryogenesis or encompasses all the facets of embryogenesis. Is this haustorium transient or persistent in the mature seed? To fill these lacunae two taxa from Cruciferae are investigated during seed development and maturation.

2. Materials and Methods

Flowers and fruits of *Iberis amara* L. and *Alyssum maritimum* Lam. grown in the Botanical Garden of Department of Botany, University of Delhi, Delhi were fixed in FAA (formalin-aceto-alcohol). The dehydration

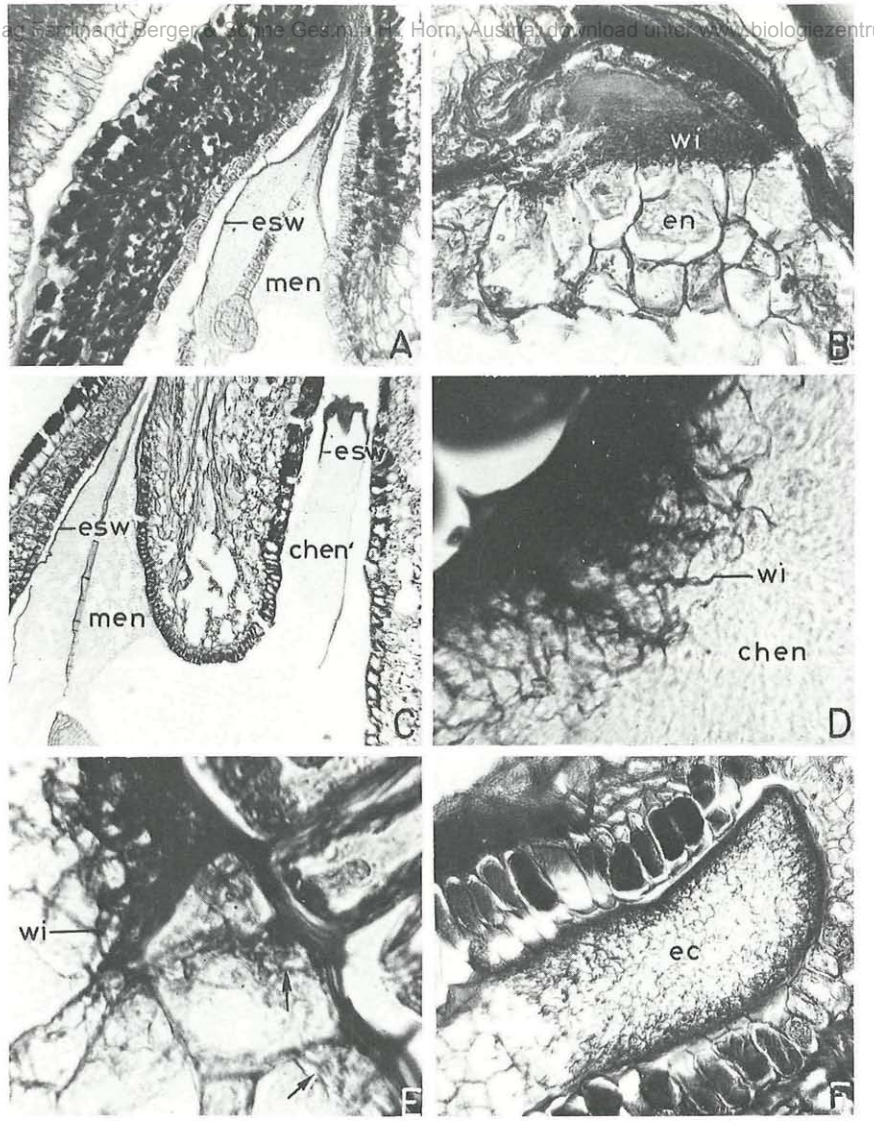

Plate 1

Plate 1 A, B. *Alyssum maritimum*, longitudinal sections of seeds (PAS-reaction) at globular proembryo and mature seed stages, respectively to reveal PAS-positive embryo sac wall (esw) enclosing micropylar portion of endosperm (men) and endosperm (en) at the chalazal end. Wall ingrowths (wi) are observed at the chalazal end of the embryo sac (A ×180; B ×340).

C—F. *Iberis amara*, longitudinal sections of seeds (PAS-reaction).

C. Seed at heart-shaped stage of embryo. PAS-positive embryo sac wall (esw) enclosing micropylar (men) as well as the chalazal (chen) portions of the endosperm is noteworthy (×98).

D. Portion of seed during maturation to show modified thick, PAS-positive embryo sac wall separating chalazal portion of endosperm (chen) from integumentary cells. The wall ingrowths (wi) stain deeply with PAS-test (×888).

E. Part of seed at a later stage. The modified embryo sac wall looses its staining capacity as it becomes *pro parte* endosperm cell. The unstained wall ingrowths (wi) can be seen in a few newly formed cells (arrows) (×888).

F. Sector from mature seed to show endosperm caecum (ec) covered with a web of ingrowths at the chalazal end of the embryo sac (×888)

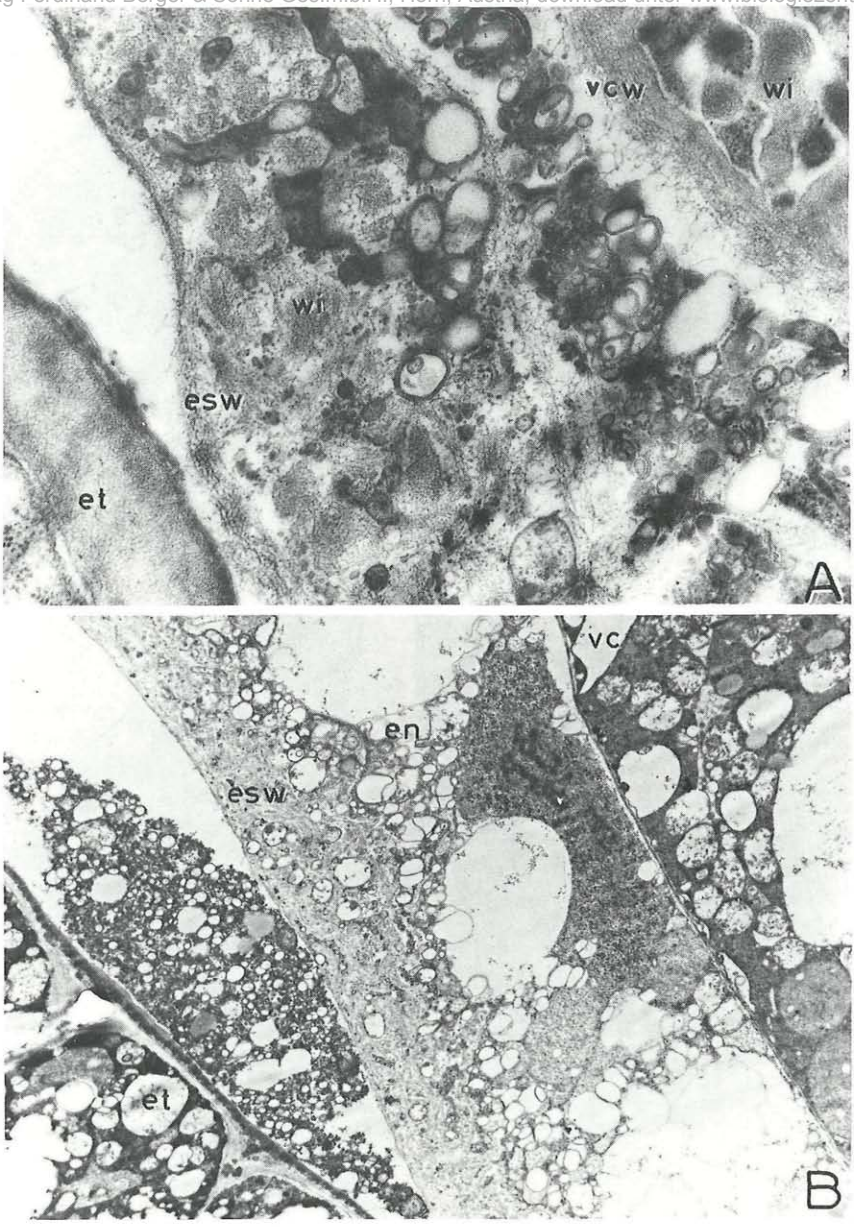

Plate 2

Plate 2. A, B. *Alyssum maritimum*, electron micrographs, longitudinal sections of seed at heart-shaped embryo stage.

A. Extreme micropylar end showing endothelium (et), embryo sac wall (esw) with wall ingrowths (wi) into the endosperm cytoplasm and modified vesicular cell wall (vcw) (×28.000).

B. Portion of endothelium (et), degenerated nucellar mass, embryo sac wall (esw), endosperm (en) and middle region of the vesicular cell (vc). Extensive labyrinth of wall projections from embryo sac wall is noteworthy. Wall of basal cell at this level is smooth (×4.000)

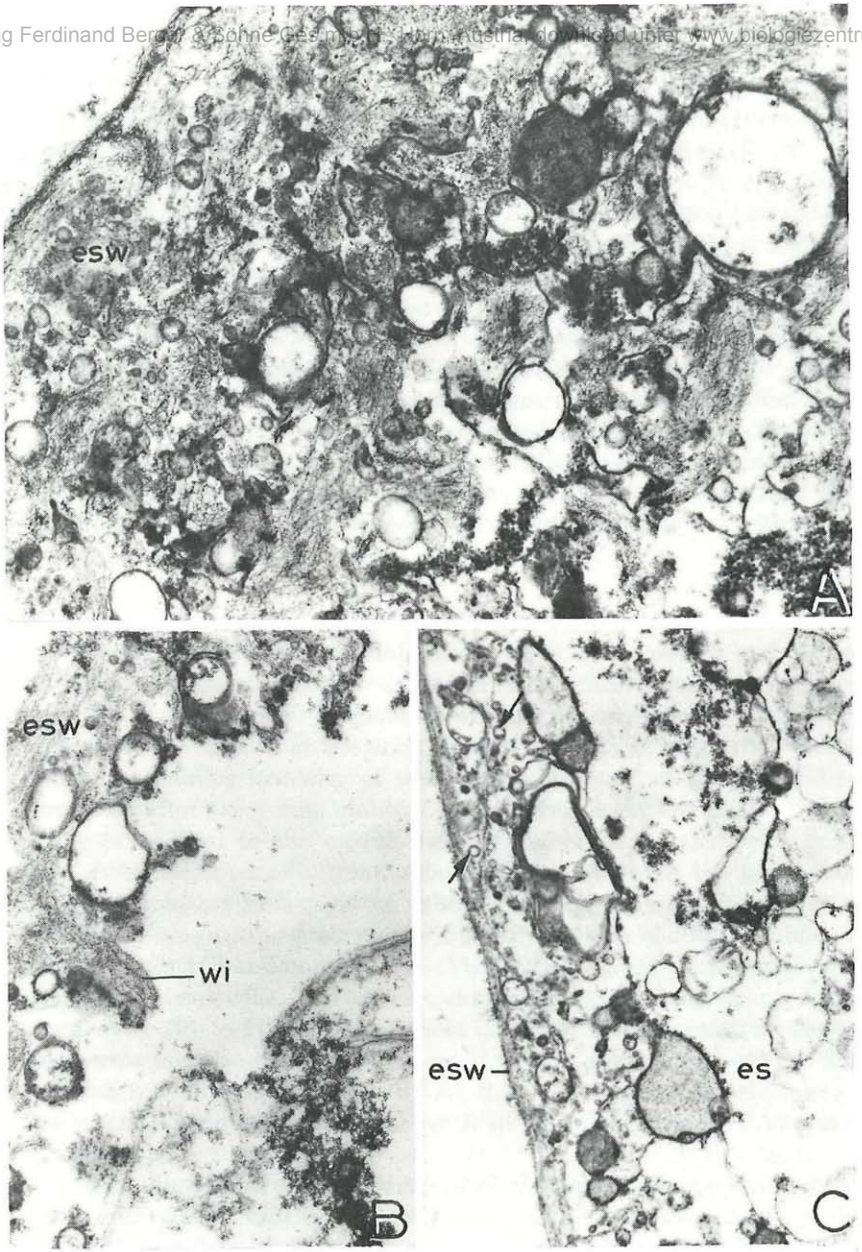

Plate 3

Plate 3 A—C. *Alyssum maritimum*, electron micrographs, longitudinal sections at heart-shaped stage of embryo.

A. Portion of embryo sac wall (esw) at the level of middle suspensor cells. Wall ingrowths are large, branched and anastomosed to form network. Numerous SER cisternae are seen associated with these ingrowths (× 20.000).

B. Part of embryo sac wall (esw) at the level of suspensor cells close to embryo proper. Simplicity of wall ingrowth (wi) is noteworthy (× 20.000).

C. Portion from the central region of the embryo sac (es). The embryo sac wall (esw) is smooth and associated with numerous SER cisternae (arrows) (× 12.000)

was done in either tertiary butyl alcohol or ethanol xylene series and infiltration and embedding in paraffin wax. Sections were cut at 12 μm.

Insoluble polysaccharides were localised with periodic acid Schiff's reaction (JENSEN 1962). Reversible acetylation, bromination and aldehyde blockade by dimedone (FEDER & O'BRIEN 1968) were done to check the specificity of PAS-reaction.

For electron microscopy, seeds of *A. maritimum* were fixed at heart-shaped embryo stage. Fixation of material was done in 3% gluteraldehyde solution in 0.05 M sodium cacodylate buffer at pH 7.2 for 3 hr at 24° C. Rinsed in buffer, postfixed in osmium tetroxide, washed in buffer, dehydrated in acetone series, infiltrated and embedded in Epon-Araldite mixture (MOLLENHAUER 1964). The ultrathin longitudinal sections were stained with 2% uranyl acetate and 0.25% lead citrate (VENABLE & COGGESHALL 1965). Electron microscopic observations were made with Siemens Elmiskop I operated at 80 KV.

3. Observations

During pro-globular proembryo stages in *Iberis amara* and *Alyssum maritimum*, embryo sac wall stains well with PAS-reaction at micropylar end. By the attainment of globular proembryo stage, positive reaction for PAS is observed both at the micropylar (Plate 1 A) as well as the chalazal end. The staining intensity of the embryo sac wall, at the chalazal end, increases after the young globular proembryo stage. The endothelial cells lying juxtaposed to the modified embryo sac wall at micropylar end do not show initiation of accumulation of tannin. In both the taxa studied this wall remains PAS-negative in the central portion of the embryo sac.

In *I. amara*, the embryo sac wall adjacent to chalazal proliferating tissue (Plate 1 C) and endothelium (Plate 1 D) is thick, deeply-stained and shows wall ingrowths. The extreme chalazal portion of endosperm remains coenocytic (Plate 1 C). At about final stages of seed maturation, the wall ingrowths increase in length, branch profusely, anastomose and are incorporated to form cell walls (Plate 1 E). Such wall ingrowths, which are *pro parte* endosperm cell walls, show little affinity toward PAS-reaction (Plate 1 E).

In mature seed, at the extreme chalazal end, the finger-like ingrowths from embryo sac wall branch profusely but do not become *pro parte* of endosperm cell walls (Plate 1 F). These ingrowths cover half the length of endosperm caecum from both sides and anastomose in the centre (Plate 1 F). The cell walls in transition zone between the cellular region of endosperm and wall ingrowths in the coenocytic portion stain with similar intensity. The wall ingrowths persist in mature seed, they degenerate gradually but can be seen up to two days after soaking of seeds.

Unlike *I. amara*, embryo sac wall in *A. maritimum* does not form projections prominent enough to be resolved under light microscope. The

embryo sac till heart-shaped stage of embryo shows PAS-positiv waell at the micropylar end (Plate 1A). The extreme chalazal end of the embryo sac wall forms conical structure which stains deeply with PAS-reaction. During seed maturation, the coenocytic chalazal portion of endosperm becomes cellular but wall ingrowths are observed at the extreme chalazal end (Plate 1B). In *A. maritimum* wall ingrowths do not participate directly in the compartmentalisation of this portion of endosperm.

Because of the advantage of smaller size as compared to *I. amara* and to confirm that the PAS-positive embryo sac wall shows modifications, the seeds of *A. maritimum* were scrutinised with the help of electron microscope.

The embryo sac wall is a continuous covering and acquires horse-shoe shape at the heart-shaped stage of embryo. This wall is closely appressed to the inner most layer of the inner integument (Plate 2A). A conspicuous feature of embryo sac wall is the presence of wall projections at micropylar (Plate 2A) and chalazal ends. The length and complexity of wall projections, at the micropylar end, is maximum at the level of basal cell (Plate 2A, B) and suspensor. The embryo sac wall, at the level of embryo proper, shows simple and unbranched wall projections associated with many smooth endoplasmic reticulum cisternae (Plate 3B). Mitochondria are also observed in abundance in association with wall ingrowths. At a level below embryo proper and in the centre of embryo sac, the wall separating endosperm from endothelium is smooth and without any projections but large number of SER cisternae are observed in close association with this wall (Plate 3C). A cuticular layer, present between endothelium and embryo sac wall, is not observed at the extreme micropylar end of embryo sac.

The wall projections are electron translucent and contain a few fibrillar stands (Plate 3A). These projections are associated with numerous cisternae of SER sectioned at various planes (Plate 3A, B). The difference in the structural pattern of wall ingrowths from embryo sac wall into the endosperm and the vesicular cell wall is well-defined (Plate 2A).

4. Discussion

Transfer cells are known to occur in numerous taxa and in extreme diverse anatomical situations. Their distinctive wall-membrane apparatus is always known to be associated with intensive short distance transport (Gunning & Pate 1969, 1974). Numerous studies on the occurrence of transfer cells indicate that there exists a morphological, temporal and spatial relationship between the degree of activity of transfer cells and the extent of development of their wall ingrowths (Gunning & Pate 1969).

In *I. amara* (present work) wall ingrowths at the chalazal end are prominent only after heart-shaped embryo stage. Prior to this stage, embryo sac shows thick, uniform and PAS-positive wall at the chalazal end. With the nuclear alignment at the periphery of embryo sac and eventual cellu-

larisation at the micropylar end of the endosperm, the absorptive activity at the chalazal end increases. Thus the time of organization of wall ingrowths indicates that the chalazal haustorium is active only during late embryogenesis. However, in Cucurbitaceae, the chalazal haustorium is active during early embryogenesis (CHOPRA & SACHAR 1963).

In *I. amara* and *A. maritimum* the coenocytic chalazal portion of the Nuclear endosperm is not delimited from the micropylar portion of the endosperm by wall. The wall ingrowths that develop at the chalazal end are involved with intensive transport of metabolites. SCHULZ & JENSEN (1974) reported wall projections at globular and heart-shaped embryo stages in *Capsella bursa-pastoris*. They further postulated that these ingrowths play an active role in absorption and translocation of metabolites secreted by the chalazal proliferating tissue. In both *I. amara* and *A. maritimum* the wall ingrowths at the chalazal end may facilitate the absorption of degraded chalazal nucellar (proliferating) tissue and/or the substances translocated by the persistent nucellar tissue (VIJAYARAGHAVAN & PRABHAKAR, unpublished). Our views support the observations of SCHULZ & JENSEN (1971, 1974).

In *I. amara* such wall projections play a dual role. In addition to the absorption of metabolites, they also play an active and direct role in dividing the coenocytic endosperm into compartments. This type of cell wall formation without the coupling of nuclear divisions is interesting. In this process of cellularisation more than one nuclei are engulfed by free wall ingrowths. It is all the more interesting that two types of *modus operandi* of cellularisation exist in these two taxa of the Cruciferae — one involving coupling of karyokinesis with cytokinesis (micropylar portion) and the other involving de-coupling of the above process. Such method of compartmentalisation of endosperm in the Nuclear type of ontogeny is interesting.

Electron microscopic studies in *A. maritimum* show labyrinth of wall ingrowths toward the endosperm at the micropylar end of the embryo sac. The present observation supports the earlier interpretation that wall ingrowths facilitate the absorption of metabolites from the ovular tissue. Such wall ingrowths into the endosperm cytoplasm are reported in *Linum usitatissimum* (VAZART & VAZART 1966), *Pisum sativum* (MARINOS 1970), *Helianthus annuus* (NEWCOMB & STEEVES 1971), *Lobelia dunnii* (TOROSIAN 1971), *Hibiscus* spp. (ASHLEY 1975), *Gossypium hirsutum* (SCHULZ & JENSEN 1977), *Haemanthus katherinae* (NEWCOMB 1978) and *Phaseolus coccineus* (YEUNG & CLUTTER 1978).

In *I. amara* and *A. maritimum* (present work) interestingly, cutinized layer of the embryo sac is absent at the micropylar end and the integumentary cells lining this end are also nontanniniferous. This reveals that, in addition to the chalazal end functioning in transport of metabolites, the channelling of nutrients from the integumentary/nucellar cells to the female gametophyte takes place at different points. In *Helianthus annuus* it occurs

at micropylar end (NEWCOMB 1973); in *Jasione montana* at submicropylar region (BERGER & ERDELSKA 1973) and in *Bellis perennis* at the chalazal end (ENGELL & PETERSEN 1977). The cuticular layer between endothelium and embryo sac is interrupted at various places and thus it is probable that the endothelium transports nutrients through these interceptions (BERGER & ERDELSKA 1973). The nutritional interrelationships that exist between different parts of the female gametophyte are interesting. The "filiform apparatus" of synergids constitutes the most common example of wall ingrowths (VAN DER PLUIJM 1964, UENSEN 1965, VAZART & VAZART 1966, VAN WENT & LINSKENS 1967, DIBOLL 1968a, SCHULZ & JENSEN 1968, VIJAYARAGHAVAN *et al.* 1972) which aid in increase of absorptive surface area. Wall ingrowths are also reported in antipodal cells (VAZART & VAZART 1966, VAZART 1968, 1969, DIBOLL & LARSON 1966, DIBOLL 1968b, RIFOT 1973); in the last suspensor cell of the embryo popularly called as "basal cell" (SCHULZ & JENSEN 1969, NEWCOMB & FOWKE 1974, PRABHAKAR 1979) and in other suspensor cells (CLUTTER & SUSSEX 1968, SCHNEPF & NAGL 1970, SIMONCIOLI 1974, NAGL 1976, YEUNG & CLUTTER 1978, 1979). Association of wall ingrowths with aleurone layer and endothelium is also known (GUNNING & PATE 1974).

It is clear that wall modifications are found in situations where adverse surface volume relationships exist between donor and receptor compartments. In *I. amara* and *A. maritimum* (present work) circumstantial evidences prove that nutrients enter the embryo sac both through the micropylar and the chalazal ends. The embryo sac wall in the central portion is smooth but is associated with large number of cisternae of SER. So the possibility of general surface absorption/translocation cannot be totally ruled out as SER are known to be associated with the translocation. If static electron micrographs and light microscope photomicrographs are indicators of the dynamics of cell/tissue concerned, then it is possible to conclude that wall ingrowths observed at specific points of micropylar and chalazal ends of the embryo sac and in special cells of female gametophyte and basal cell of the embryo do play an active role in translocation.

Acknowledgement

Dr. (Mrs.) Kumkum PRABHAKAR acknowledges the award of Research Associatenship by the Council of Scientific and Industrial Research, New Delhi.

References

ASHLEY T. 1975. Fine structure of early endosperm development in *Hibiscus*. — Caryologia 28: 63—71.

BERGER C. & ERDELSKA O. 1973. Ultrastructural aspects of the embryo sac of *Jasione montana* L. cell walls. — Caryologia 25 (Suppl.): 109—120.

CHOPRA R. N. & SACHAR R. C. 1963. Endosperm. — In: Recent Advances in the Embryology of Angiosperms (Ed. P. MAHESHWARI), International Society of Plant Morphologists, Dept. of Botany, Univ. of Delhi.

CLUTTER M. E. & SUSSEX I. M. 1968. Ultrastructural development of bean embryo cells containing polytene chromosomes. — J. Cell Biol. 39: 26a.

DIBOLL A. G. 1968a. Histochemistry and fine structure of the pollen tube residue in the megagametophyte of *Zea mays*. — Caryologia 21: 91—95.

— 1968b. Fine structural development of the megagametophyte of *Zea mays* following fertilisation. — Am. J. Bot. 55: 787—806.

— & LARSON D. A. 1966. An electron microscopic study of the mature megagametophyte in *Zea mays*. — Am. J. Bot. 53: 391—402.

ENGELL K. & PETERSEN G. B. 1977. Integumentary and endothelial cells of *Bellis perennis*. Morphology and histochemistry in relation to the developing embryo sac. — Bot. Tidsskr. 71: 237—244.

FEDER N. & O'BRIEN T. P. 1968. Plant microtechnique: Some principles and new methods. — Am. J. Bot. 55: 123—142.

GUNNING B. E. S. & 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 (Ed. A. W. ROBARDS). — Maidenhead, England.

JENSEN W. A. 1962. Botanical Histochemistry, Principle and Practice. — San Francisco, USA.

— 1965. The ultrastructure and histochemistry of the synergids of cotton. — Am. J. Bot. 52: 238—256.

MAHESHWARI P. 1950. An Introduction to the Embryology of Angiosperms. — New York.

MARINOS N. G. 1970. Embryogenesis of the pea (*Pisum sativum*). I. The cytological environment of the developing embryo. — Protoplasma 70: 261—279.

MOLLENHAUER H. H. 1964. Plastic embedding mixtures for use in electron microscopy. — Stain Technol. 39: 111—114.

NAGL W. 1976. Early embryogenesis in *Tropaeolum majus* L. ultrastructure of the embryo-suspensor. — Biochem. Physiol. Pflanz. 170: 253—260.

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

— 1978. The development of cells in the coenocytic endosperm of the African blood lily *Haemanthus Katherinae*. — Can. J. Bot. 56: 483—501.

— & FOWKE L. C. 1974. *Stellaria media* embryogenesis: The development and ultrastructure of the suspensor. — Can. J. Bot. 52: 607—614.

— & STEEVES T. A. 1971. *Helianthus annuus* embryogenesis: Embryo sac wall projections before and after fertilization. — Bot. Gaz. 132: 367—371.

PRABHAKAR, Kumkum 1979. Histochemical and ultrastructural studies in some *Cruciferae*: Zygote to seedling. Ph. D. Thesis, University of Delhi, Delhi.

RIFOT M. 1973. Evolution structurale du pole chalazien du sac embryonnaire d' *Aquilegia vulgaris* en liaison avec son activite trophique. — C. r. hebd. Séanc. Acad. Sci., Paris 277: 1313—1316.

SCHNEPF E. & NAGL W. 1970. „Über einige Strukturbesonderheiten der Suspensorzellen von *Phaseolus vulgaris*". — Protoplasma 69: 133—143.

SCHULZ S. P. & JENSEN W. A. 1971. *Capsella* embryogenesis: The chalazal proliferating tissue. — J. Cell Sci. 8: 201—227.

— — 1974. *Capsella* embryogenesis: The development of the free nuclear endosperm. — Protoplasma 80: 183—205.

— — 1977. Cotton embryogenesis: The early development of the free nuclear endosperm. — Am. J. Bot. 64: 384—394.

SCHULZ S. P. & JENSEN W. A. 1969. *Capsella* embryogenesis: The suspensor and the basal cell. — Protoplasma 67: 139—163.

SCHULZ S. R. & JENSEN W. A. 1968. *Capsella* embryogenesis: The synergids before and after fertilization. — Am. J. Bot. 55: 541—552.

SIMONCIOLI C. 1974. Ultrastructural characteristics of "*Diplotaxis erucoides* (L.) DC." suspensor. — G. Bot. ital. 108: 175—189.

TOROSIAN C. D. 1971. Ultrastructural study of endosperm haustorial cells of *Lobelia dunnii* GREENE (*Campanulaceae, Lobelioideae*). — Am. J. Bot. 58: 456—457.

VAN DER PLUIJM J. E. 1964. An electron microscopic investigation of the filiform apparatus in the embryo sac of *Torenia fournieri*. — In: *Pollen Physiology* and *Fertilization*, (Ed. H. F. LINSKENS). — Amsterdam.

VAN WENT J. & LINSKENS H. F. 1967. Die Entwicklung des sogenannten „Fadenapparates" im Embryosack von *Petunia hybrida*. — Gen. Breeding Res. 37: 51—56.

VAZART B. & VAZART J. 1966. Infrastructure du sac embryonnaire du lin (*Linum usitatissimum* L.). — Rev. Cytol. Biol. vég. 24: 251—266.

VAZART J. 1968. Infrastructure de l'ovule du lin, *Linum usitatissimum* L. — Le complex antipodal. — C. r. hebd. Séanc. Acad. Sci., Paris 266: 211—213.

— 1969. Organization et ultrastructure du sac embryonnaire du lin (*Linum usitatissimum*). — Rev. Cytol. Biol. vég. 32: 227—240.

VENABLE J. H. & COGGESHALL R. 1965. A simplified lead citrate stain for use in electron microscopy. — J. Cell Biol. — 25: 407—408.

VIJAYARAGHAVAN M. R., JENSEN W. A. & ASHTON M. E. 1972. Synergids of *Aquilegia formosa* — their histochemistry and ultrastructure. — Phytomorphology 22: 144—159.

YEUNG E. C. & CLUTTER M. 1978. Embryogeny of *Phaseolus coccineus*: Growth and microanatomy. — Protoplasma 94: 19—40.

— — 1979. Embryogeny of *Phaseolus coccineus*: the ultrastructure and development of the suspensor. — Can. J. Bot. 57: 127—136.