DISCUSSION

Morphology:

Collection and identification of cultivars and wild species are the first step in a breeding programme. *Sesamum* germplasm collection has been undertaken all over the world which includes 1645 accessions (Ashri, 1989). *S. radiatum*, *S. alatum* and *Ceratotheca triloba* and four unknown entries are the few wild species. In 1992, NBPG- Japan international cooperation Agency, reported 160 cultivated *Sesame* samples, two weedy and one wild species from Karnataka, Maharashtra, Madhya Pradesh, and Uttar Pradesh. In India under the IBPGR Project 554 accessions were made from Maharashtra, Himachal Pradesh, Uttar Pradesh, Kerala, and Tamil Nadu which consist of *Sesamum indicum* var. *malabaricum*, *S. laciniatum*, *S. radiatum* (IBPGR - Newsletter, 1993). In the present investigation 5 wild species have been collected. However all the collections do not even cover even 1/4 of the *Sesamum*’s total species.

Unfortunately the situation on the taxonomic status of the genus *Sesamum* remains the same since the time of Joshi’s (1961) monograph on *Sesamum*. Revision of genus using cytotaxonomy is necessary.

The taxonomy of *Sesamum malabaricum* (= *S. malayanum*) is controversial, often disputed. The species which is listed in Van Rheede’s *Hortus malabaricus* (1689) with the local name ‘car-ellu’ which means ‘Black Sesame’ (Nicolson et al., 1988). Description reveals that this is a seed variant of *S. orientale* and there is no species as *S. malabaricum*.

John et al., (1951) reported a variety *S. orientale* var. *malabaricum* from Malabar differentiated by the pale violet corolla with dark purple lower lip, dark purple lines along the line of dehiscence in the anther, rugose seeds and with chromosome number 2n = 26. This species is being maintained in Tamil Nadu Agricultural University, Coimbatore for breeding purposes. In 1963, Nair reported a new species named *S. malayanum* from Punjab without referring to the species of
John et al., and also ignoring the above mentioned characters. But all other characters coincide with John et al's species.

Later, Mitra and Biswas (1983) reported *S. mulayanum* from Bengal and mentioned the above characters as additional information and they reported the chromosome number $2n = 26$. They have referred to a species *S. indicum* var *malabaricum* for which no reference had been quoted. This variety could be the same one, referred by Nicolson et al., (1988).

Bennett (1974) reported that *S. mulayanum* and *S. indicum* are similar in appearance but can be easily distinguished by the seeds which are reticulate in the former and smooth in the latter. Babu (1977) and Kulkarni (1988) observed that *S. mulayanum* appears to be a seed variant of *S. orientale* in which the surface of the seed varies from smooth to rugose and concluded, that experimental studies are needed to throw light on the relationship among two. But Bhandari (1990) and V.J. Nair (personal communication) opined that *S. mulayanum* is distinct species with seeds having raised reticulations and rectangular cross section.

The present investigation based on the specimens available in Tamil Nadu Agricultural University has also proved that *S. orientale* and *S. mulayanum* are two different species with same chromosome number ($2n = 26$), because of the following differences in the morphology.

<table>
<thead>
<tr>
<th><em>S. orientale</em></th>
<th><em>S. mulayanum</em></th>
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</thead>
<tbody>
<tr>
<td>1. Leaves: sparsely hairy</td>
<td>Densely tomentose</td>
</tr>
<tr>
<td>2. Flowers: white, often</td>
<td>Violet</td>
</tr>
<tr>
<td>tinged with pale pink</td>
<td></td>
</tr>
<tr>
<td>3. Corolla: Lip same as other parts</td>
<td>Anterior lip deep purple</td>
</tr>
</tbody>
</table>
4. No purple dots in the throat of corolla

Fine deep purple dots are present in the throat of corolla

5. Stamens: anther white

Anther white with deep purple line along the line of dehiscence

6. Ovary oblong, subglabrous

Ovary obconical, densely pubescent

7. Seed-smooth, seed coat thin

Rugose with reticulate ridges and thick seed coat.

SEM studies on seed coat surface reveal that the seed coat of *S. orientale* has hexagonal cells with convex surface and hence it appears smooth whereas the seed coat of *S. mulayanum* reveal presence of reticulate ridges and the cells have concave surface and seed coat appears rugose. The above characteristics justify that they are two different species and can be treated separately.

Introduction of disease resistance from wild species to cultivated species is the main objective in *Sesamum* breeding. Ramanujam (1942) reported that the plants of *S. prostratum* are resistant to diseases and pests. Ramanathan (1950) reported disease and drought resistance in *S. prostratum*, *S. laciniatum*, *S. radiatum* and *S. occidentale*, *S. mulayanum* is found to be tolerant to salt (Kulkarni, 1971). In contrast to this the wild species are reported to be susceptible to any one of the diseases. *S. alatum*, *S. radiatum*, *S. prostratum* and *S. mulayanum* are susceptible to phyllody (Ramanujam, 1944, Mazzani and Malaguti, 1952; Vasudeva, 1961 Kavathekar et al., 1973 and present investigation) *S. radiatum* is susceptible to stem rot also (See Vasudeva, 1961 and present investigation).

**Developmental Embryology**

Embryological investigations in the family Pedaliaceae are restricted to a few particular genera viz. *Sesamum*, *Pedaliam* and *Martynia*. The members of the family vary greatly in their habit, morphology and chromosome numbers. The chromosome
numbers of Indian Pedaliaceae have been summarised by Virendrakumar and Subramaniam (1986). It ranges from 2n = 16 to 2n = 64. *Pedalium murex* (2n = 16) is a prostrate succulent herb, *Sesamum prostratum* (2n = 32) and *S. laciniatum* (2n = 32) are prostrate herbs. All the other members are erect herbs and *Trapella* is an aquatic herb. Similarly they vary in the placentation and in the position of the ovary and fruit morphology.

In spite of such wide variations, the embryological features of the members of the family remain uniform (see review). However present investigations on *Sesamum alatum*, *S. laciniatum* and *S. radiatum* reveal some features which are new and contrast to previous reports.

Earlier embryological work has been ably reviewed by Davis (1966). Reports on the development of the anther wall is lacking except for that of Singh (1963) who reported that in *Martynia diandra* the outer secondary parietal layer divides to contribute the wall layers. But in the present investigation it is observed that both the secondary parietal layers divide and contribute to the wall layers confirming to the basic type. The tapetal cells are binucleate and are highly vacuolated in the wild species of *Sesamum* whereas they are bi-, or multinucleate and multinucleolate in other members. Singh (1960) reported that the endothecium becomes two layered in some places in *S. indicum* as in *S. radiatum* (present investigation). Dimorphic tapetal cells and dyads of *S. laciniatum*, degeneration of spores in a tetrad in *S. alatum* are reported in the present investigation.

Division of the female archesporial cell observed in one of the ovules of *S. alatum* is a rare feature for a tenuinucellate ovule. Multiple archesporial cells have been reported in *S. indicum* (Nohara, 1934; Srinivasan, 1942; Hanawa, 1953; and Singh, 1960). *P. murex* and *M. diandra* (Singh, 1963; 1964) whereas in *S. alatum*, *S. laciniatum* and *S. radiatum* only one archesporial cell is observed in the ovules.

Presence of endothelium is a common feature in the family which has not been paid much attention. In the present investigation it is observed that the endothelial layer is less distinguished at embryo sac stage, becomes more prominent
after fertilization and on degeneration its contents are deposited as a lining layer of the embryo sac.

**Division** of the zygote is delayed until the formation of endosperm. The time for the division of the zygote varies with the species.

- **S. indicum** (Nohara, 1934; Joshi, 1961) - 30 hrs.
- **S. prostratum** (Dadlani, 1958) - 132 hrs.
- **S. radiatum** (Dadlani, 1958) - 144 hrs.

In the present investigation it is observed that the primary endosperm nucleus divides immediately after fertilization in all the species whereas the division of the zygote shows variation. The zygote divides within 48 hrs in **S. alatum** and it takes 8 to 9 days in **S. laciniatum** and **S. radiatum**.

Another interesting feature is the elongation of the zygote and the development of the micropylar haustorium. Singh (1960) reported that in **S. indicum** the zygote elongates to a considerable length and pushed deep into the tissues of the endosperm. In contrast to this it is observed that in **S. alatum**, **S. laciniatum**, and **S. radiatum** the zygote elongates only to a certain extent but not as reported earlier. Instead there is an extension of the micropylar portion of the ovule beyond the zygote so that the embryo appears to be in the centre of the embryo sac.

Singh (1960) differentiated the endosperm of **S. indicum** into 3 parts based on the meristematic activity of the cells as micropylar, central and chalazal. In the present investigation it is observed that it can be divided into 5 parts based on the staining intensity and the nature of the cells as described in the observation. The micropylar haustorium itself consists of 3 regions where the I and III regions staining intensely with dense cytoplasm and the II and IV regions consist of enlarged vacuolated cells with faint cytoplasm. V region is chalazal endosperm which consists of 4 cells (present investigation).
It is stated earlier (Singh, 1960) that the micropylar haustorium differentiates after the degeneration of chalazal haustorium. In contrast to this, it is observed in the present investigation that both the processes occur simultaneously. Micropylar haustorium degenerates in all species including those species under present investigation, but in *Pedalium murex* it persists (Singh, 1963).

From the above short discussion it is evident that except few, most of the genera remain uninvestigated, and in the few studied earlier reinvestigations are still required which may open new avenues in the embryological status of the family, and also help solving taxonomic problems of *Martynia* and *Trapella*.

*S. alatum* differs from other species in the presence of palmate leaves in the lower region and the seeds are winged. But the chromosome number is the same $2n = 26$, as that of *S. orientale*. *S. laciniatum* differs drastically by its prostrate habit with $2n = 32$ and *S. radiatum*, the Nigerian species has $2n = 64$, with rough serrate leaves. Embryological investigation on these wild species show very minor differences among them. Hanawa (1953) did not find any difference in the development of megaspore and embryo between diploid and tetraploid strains of *S. orientale* except in the rate of growth which is slower in the tetraploid strain. *S. alatum* resembles *S. orientale* in the rapid development of the embryo, endosperm and 100% seed set. *S. radiatum* resembles *S. orientale* in the presence of well formed tapetal cells, double-layered endothecium and it differs from *S. orientale* by the presence of weak, sterile seeds and slow growth of the embryo. *S. radiatum* and *S. laciniatum* resemble each other in the rate of embryo development. *S. laciniatum* stands unique among the 4 species by its dimorphic tapetum, formation of dyads, ovule sterility and abscission of capsules at maturity. As stated by Hanawa (1953) the higher chromosome number may be the reason for the slow growth of the embryo in *S. laciniatum* and *S. radiatum*. Non-directional pollen tube growth, ovule degeneration of *S. laciniatum* and weak endosperm formation, of *S. radiatum* are features which have been reported as new in this family. A common feature observed
among the 4 species of *Sesamum* is that the development of the embryos in the flowers opened on the same day do not follow the same rate of growth.

**Histochemical Embryology**

Present investigation on the histochemical embryology on the anther, ovule, embryo and endosperm of *S. orientale*, *S. alatum*, *S. laciniatum* and *S. radiatum* reveal uniform pattern of distribution of RNA, protein and insoluble polysaccharides at similar stages in the development with slight variation in *S. orientale* and *S. radiatum*. There is an accumulation of PAS-positive grains in the wall layers of anthers before the formation of fibrous thickenings in the endothecium in *S. orientale* and *S. radiatum*. Similarly the amount of RNA and proteins increase at pre-globular stage of the embryo which is delayed a little in other two species. The synergids of *S. radiatum* contain proteins than the other three species. Histochemical studies have been carried out in the anthers of *S. indicum* (Anurag Titov, 1984) and *Martynia diandra* (Hegde, 1986) which showed high accumulation of RNA, protein, insoluble polysaccharides and ascorbic acid in the tapetal cells as in the present investigation.

In *Sesamum* the male archesporial cells are distinct by their rich content of RNA, proteins, low cytoplasmic polysaccharides as in *Rauvolfia serpentina* (Panchaksharappa and Koppar, 1972), *Farsetia hamiltonii*, *Eruca sativa* (Prasad, 1977a) and *Atriplex repens* (Dulcy, 1983). In *Sesamum* this status continues till the differentiation of wall layers and sporogenous cells and their intensity of RNA, protein and cytoplasmic polysaccharides decrease in the wall layers thus indicating their utilization during differentiation. But the cell walls are PAS-positive. With maturity, in *S. orientale* and in *S. radiatum*, PAS positive grains appear in the wall layers before the formation of endothecial thickenings. Similar PAS-positive grains are observed in the wall layers of *Zea mays*, *Paspalum scrobiculatum* (Panchaksharappa and Rudramuniappa, 1974, 1975) and *Smilax macrophylla* (Panchaksharappa and Syamasundar, 1974a), *Carica papaya* (Rudramuniappa et al., 1987) before endothecial thickenings.
It *Sesamum* tapetum has high RNA, protein and insoluble polysaccharides in the peripheral cytoplasm indicating its nutritional role. Panchaksharappa and Rudramuniappa (1974, 1975a) reported the presence of PAS-positive grains and low amount of cytoplasmic polysaccharides in the tapetal cells of *Zea mays, Paspalum scrobiculatum* and *Sorghum vulgare*. The Ubisch granules of *Atriplex* (Dulcy, 1983) react positively for PAS reaction. The Ubisch granules of the species of *Sesamum* stain bluish green with Azure B as in *Euphorbia pulcherrima* (Rudramuniappa and Annigeri, 1985) and react negatively for protein and polysaccharides. The Ubisch granules of *Carica papaya* stain for sporopollenin (Sheel and Bhandari, 1990).

In *Sesamum* sporogenous cells have moderate cytoplasmic insoluble polysaccharides which increase in the microspore mother cells. Similar observations have been reported in *Smilax macrophylla* (Pachaskharappa and Syamasundar, 1974a) and *Atriplex repens* (Dulcy, 1983). Polysaccharide contents decrease prior to meiosis in *Raphanus sativus* (Panchaksharappa and Koppar, 1975), *Coronopus didymus* (Chauhan, 1979), *Sorghum* vulgare (Panchaksharappa and Rudramuniappa, 1975a) and *Atriplex repens* (Dulcy, 1983). RNA and proteins are at low level in the sporogenous cells of *Sesamum* as in *Zea mays* (Panchaksharappa and Rudramuniappa, 1974). But the sporogenous cells as well as the pollen mother cells of *Smilax macrophylla* (Panchaksharappa and Syamasundar, 1974a), *Raphanus sativus* (Panchaksharappa and Koppar, 1975), *Nigella demascena* (Bandari et al., 1976), *Luffa acutangula* (Panchaksharappa and Shelke, 1977), *Farsetia hamiltonii*, *Eruca sativa* (Prasad, 1977a), *Capsicum annuum* (Nalini, 1979) and *Atriplex repens* (Dulcy 1983) have rich cytoplasmic and nucleolar RNA and proteins.

In *Sesamum* the amount of proteins and polysaccharides increase in the microspore mother cells before meiosis and remain constant even in the microspore tetrads. A slight reduction of RNA is observed in the mother cells before meiosis as in *Zea mays* (Panchaksharappa and Rudramuniappa, 1974). In *Calotropis procera* there is an initial decrease of RNA and protein in the sporogenous cells followed by a rise before meiosis in the pollen mother cells (Vijayaraghavan and Cheema, 1978).
In *Sesamum* the additional callose wall of the microspore mother cell reacts faintly for PAS. In contrast to this the callose layer is PAS-positive in millets (Panchaksharappa and Rudramuniappa 1972a), *Zea mays* (Panchaksharappa and Rudramuniappa, 1974), *Rhoeo discolor* (Albertini, *et al.*, 1981) and *Carthamus tinctorius* (Bhandari and Sharma, 1983). In *Kalanchoe* (Rudramuniappa and Annigeri, 1984) and *Guizotia* (Rudramuniappa, 1991) the microspore mother cell wall contains ascorbic acid. The callose wall is rich in polysaccharides and proteins in *Iphigenia pallida* (Panchaksharappa and Syamasundar, 1974b). Rich cytoplasmic polysaccharides, RNA and proteins are observed in the dyads and tetrads of *Smilax macrophylla*, *Iphigenia pallida*, (Panchaksharappa and Syamasundar, 1974a,b), *Atriplex repens* (Dulcy, 1983) and in *Sesamum* (present investigation). Mature pollen grains have rich RNA and polysaccharides, but low levels of proteins in *Sesamum*. All the macromolecules are at low level in *Setaria italica* and *Zea mays* (Panchaksharappa and Rudramuniappa, 1972a, 1974). Starch grains are reported in the mature pollen grains of *Pyrostegia* (Nanda, 1975; Aswath *et al.*, 1989), *Coronopus didymus* (Chauhan, 1979), *Nicotiana tabacum* (Lunyeva *et al.*, 1970) and *Datura alba* (Bhatia and Chopra, 1978).

Microsporogenesis involves cell division and differentiation which needs energy through biochemical substances. It is a well known fact that RNA plays the key role in the synthesis of proteins which in turn involved in various metabolic activities during cell division. The polysaccharides release ATPs which are essential for the actively dividing cells for the synthesis of RNA (Mahler and Cordes, 1971). Polysaccharides also form the building materials for the newly formed cells. Based on this concept it can be interpreted that the dividing cells such as archesporial cells and microspore mother cells are rich in RNA, protein, thin PAS-positive wall and rich cytoplasmic polysaccharides. But the wall layers before differentiation contain higher amount of macromolecules which decline after differentiation clearly indicating that the metabolic activities slow down and thereafter they not only give mechanical protection, but also serve as storage centres for starch and lipids. These
substances will be mobilized later for the development of pollen and fibrous thickenings of the endothecium (see Panchaksharappa et al., 1985). The decline of polysaccharides and PAS positive granules after meiosis and endothecial formation in many plants including present investigation confirms this view.

The tapetum is the only nutritive layer with high amount of RNA, protein and polysaccharides, and its nutritive role is suggested by the close proximity with microspore mother cells and its malfunction is a primary cause for the initiation of abortive process in pollen formation in the male sterile anthers of various taxa (Rudramuniappa et al., 1985). Formation of Ubisch granules immediately after tapetal degeneration suggest their role in the formation of pollen wall. The microspores represent the gametophytic generation behaving as an independent unit. Presence of proteinaceous bodies, storage starch, and RNA indicates that they can serve as source of energy during the division of pollen nucleus, germination and tube growth upto fertilization. Similar interpretation on the nature of pollen storage materials has been given by Rudramuniappa et al., (1987) as well.

The ovule primordium as in anther primordium has rich RNA, protein and PAS positive wall (present investigation). Storage starch is reported in the members of Gramineae (Panchaksharappa and Rudramuniappa, 1972b). Rich RNA and protein in the archesporial cells of Sesamum decrease when it develops into megaspore mother cell. But the contents of RNA and proteins remain as such in Stellaria media (Pritchard, 1964a), Dipcadi montanum Panchaksharappa and Syamasundar, 1975) and Atriplex repens (Dulcy, 1983). The archesporial cell of Sesamum has PAS positive cytoplasm as in Trigonella foenum-graceum (Panchaksharappa and Hegde, 1971) and Dioscorea bulbifera (Panchaksharappa and Ranaware, 1978) whereas low level of polysaccharides are reported in Atriplex repens (Dulcy, 1983).

In the present investigation it is observed that RNA, protein and cytoplasmic polysaccharides are reduced in the megaspore mother cell, tetrads and in the embryo sac. Low RNA and proteins in Sesamum agree with the results of Prichard (1964a)
in *Stellaria media*, Prasad (1977b) in *Eruca sativa*, *Farstia hamiltonii* and Aswath *et al.*, (1989) in *Pyrostegia venusta*. In the embryo sac also proteins are at moderate level in the egg, polar nuclei and antipodals and less proteins in the synergids of *Stellaria media* (Pritchard 1964a) and Mimusops hexandra (Jindal and Paliwal, 1981).

In *Sesamum* the egg and synergids have moderate cytoplasmic polysaccharides in contrast to less cytoplasmic polysaccharides in *Allium cepa* (Syamasundar and Panchaksharappa, 1975), *Dipcadi montanum* (Panchaksharappa and Syamasundar, 1975), *Argemone mexicana* (Bhandari *et al.*, 1980) and *Atriplex repens* (Dulcy, 1983). Synergids have less RNA and proteins (present investigation) whereas Panchaksharappa and Syamasundar (1975) reported rich cytoplasmic proteins and less RNA in the synergids of *Dipcadi montanum*.

Antipodals of *Sesamum* are rich in RNA and proteins as in *Eleusine* (Panchaksharappa and Rudramuniappa 1975b) but those of *Stellaria media* (Pritchard, 1964a) and *Dipcadi montanum* (Panchaksharappa and Syamasundar, 1975) and *Atriplex repens* (Dulcy, 1983) have low RNA and proteins.

In *Sesamum* the integumentary cells near the micropyle have high amount of starch grains which appear at megaspore tetrad stage as in *Paspalum longfolium* (Yu and Chao, 1979). The remaining cells have PAS positive walls as in *Stellaria media* (Pritchard, 1964a). Polysaccharide granules are reported in the nucellus at the chalazal end of *Zea mays* at mature embryo sac stage (Rudramuniappa and Panchaksharappa, 1976). The cells of the hypostase have less proteins and no RNA. But they are rich in *Dipcadi montanum* (Panchaksharappa and Syamasundar, 1975).

The cells of the endothelium and other integumentary cells stain alike in *Sesamum* as in *Foeniculum vulgare* (Agarwal and Gupta, 1976a). Starch grains are reported in *Alyssum maritimum* (Prabhakar and Vijayaraghavan, 1982). Immediate increase of metabolites after fertilization suggests their nutritive role during the ovule enlargement in *Sesamum* (present study).

Central cell of *Sesamum* contains high accumulation of starch as in *Capsella bursa-pastoris* (Schulz and Jensen, 1968a), members of the Gramineae
Initially the zygote is rich in RNA, protein and insoluble polysaccharides in *Sesamum*. Similar observations have been reported in many plants so far investigated but that of *Dioscorea bulbifera* (Panchaksharappa and Ranaware, 1978) and millets (Panchaksharappa and Rudramuniappa, 1972b) have low cytoplasmic polysaccharides.

The intensity of RNA, protein and polysaccharides decrease to a minimum level before the division of the zygote and remains to be the same till the embryo attains early globular stage (present observation). This is in contrast to the previous reports where RNA and proteins are at high level in 2-celled and 4-celled proembryos of *Panicum* (Rudramuniappa and Panchaksharappa, 1979). Marked differences in the contents of RNA, proteins and polysaccharides are reported between the basal and terminal cells of the proembryo in several plants studied earlier.

The globular embryo shows equally distributed high RNA and proteins in *Sesamum* as in *Lens culinaris, Myosurus minimum, Alyssum maritimum* (Rondet, 1958, 1961, 1962 respectively), *Capsella bursa-pastoris* (Schulz and Jensen, 1968b), *Petunia hybrida* (Vallade, 1970) *Limnophyton obtusifolium* (Shah and Pandey, 1978b), *Nicotianna tabacum* (Norreel, 1972) etc. In *Sesamum* the cell walls are PAS positive, cytoplasm has moderate polysaccharides and a few polysaccharide granules are seen scattered in the embryo proper at early globular stage as in *Arachys hypogaea* and *A. monticola* (Jayalakshmi, 1982).

In *Sesamum*, the polysaccharides, RNA and protein contents remain to be the same even after the differentiation of cotyledons. But in *Atriplex* ((Dulcy, 1983) polysaccharides decrease at this stage. PAS positive grains are observed in the shoot apex of *S. laciniatum* and root apex of *S.alatum*. Polysaccharide grains are observed in the cotyledons and root cap region of *Atriplex repens* (Dulcy, 1983).

Throughout the embryo development the suspensor has very low content of RNA, protein and polysaccharides in *Sesamum*. RNA is low in the embryo proper and more in the suspensor in *Farsetia hamiltonii* and *Eruca sativa* (Prasad, 1977b). The low amount of metabolites of *Sesamum* may be due to extensive micropylar endosperm haustorium.

Histochemical studies are very limited on the endosperm. The endosperm of *Sesamum* is divided into micropylar and chalazal endosperm. The 4-celled chalazal endosperm has rich RNA, protein and polysaccharides. But the micropylar endosperm shows variation in the staining intensity among different parts. The I and III regions of the haustorium contain cells with rich RNA, proteins and insoluble polysaccharides whereas the II region of the haustorium and the endosperm proper show similar faint intensity for these macromolecules. In *Arachis hypogaea* (Periasamy and Sampoornam, 1979) proteins are at low level at all stages and RNA is rich at free nuclear stage. The distribution of polysaccharides shows variation in *Sesamum* (present study). The cells at the globular stage of the embryo are uniform and contain less amount of polysaccharides. When the embryo attains heart-shaped stage the cells adjacent to the embryo degenerate and the products show high PAS positive reaction indicating the utilization by the rapidly growing embryo. Periasamy and Sampoornam (1979) reported presence of polysaccharide granules in the endosperm of *Arachis hypogaea* before wall formation.

During megasporogenesis the metabolites are at low level even in the dividing cells such as megaspore mother cell. Presence of polysaccharide granules at the micropylar region of the ovule in *Sesamum* suggests their utilization at the time of micropylar extension of the ovule. Aswath et al., (1989) suggested in *Pyrostegia venusta* that rich accumulation of starch content and low RNA and proteins indicate
decreased metabolic activity where degeneration of ovules are observed. Presence of starch in the central cell indicate their necessity during endosperm development, as Schulz and Jensen (1968a) are of the opinion that the polysaccharides stored in the egg and central cell act as a source of carbon for the embryo during early growth phase. According to Raghavan (1976) in young embryos tissue proteins predominate, but in older embryos most of the proteins are storage proteins. He also suggested that the synthesis of RNA and proteins indicate the initiation of new developmental potencies. High intensity in the globular and heart-shaped stage indicate their physiologically active stage (Shah and Pandey, 1978b).

Embryology of Interspecific Crosses

Pollen germination, pollen tube growth, ovule penetration and fertilization are critical genetically controlled events in the reproductive cycle of a plant. In interspecific crosses, inhibition or abnormalities of any one of these events lead to failure of seed set and the phenomenon is said to be sexual incompatibility. This is common among the different species having the same or different chromosome numbers. The species of Sesamum are said to be self-compatible with different chromosome numbers. In the present investigation in vivo studies on self-pollination of S. orientale, S. alatum, S. laciniatum, S. radiatum, the crosses S. orientale x S. alatum, S. orientale x S. laciniataum, S. orientale x S. radiatum and their reciprocal crosses show similar pollen germination and tube growth. Abnormalities such as inhibition of pollen germination, formation of short tubes, pollen tubes bursting at different levels of the style are observed. These abnormalities are usual signs of incompatibility reactions (de Nettancourt, 1977). In addition non-directional growth of the pollen tube is observed which according to Evans (1962) may be due to physiological repulsion of the stylar tissue.

The abnormalities observed in self-pollination suggest presence of self-incompatibility in Sesamum (present investigation). It indicates Lewis and Crowe's (1958) hypothesis that self-compatible species have evolved from self-incompatible
ones by stepwise mutation. Similar pollen germination, tube behaviour, embryo and endosperm development in all the 4 species suggest their close relationship. The variations observed in the percentage pollen germination, stylar, stigmatic inhibition and pod-set seems to have no correlation. In *Sesamum* the percentage of compatible grains must be more and hence there is 100% seed set during self-pollination. But the data on pollen germination does not help to correlate such an interpretation, because in *S. orientale*, the percentage germination is 36.7%, stigmatic inhibition is 21.9% and stylar inhibition is 17.5% where pod and seed set is 100% whereas in *S.alatum* pollen germination is 78.8 with 7% stigmatic inhibition and 6.04 stylar inhibition. Such complicated variations may be due to random selection of the pistils and incongruity within the species. Kho *et al.*, (1980) also reported arrest of pollen tubes at the levels of stigma, style or ovary and normal pollen tubes after selfing in *Cucumis* where there is no self-incompatibility.

Differences are not observed in pollen germination and tube growth in both self and cross pollinations of *Corchorus olitorius* x *C. capsularis* (Patel and Datta, 1960); self- and inter-sectional crosses of poplars (Stettler *et al.*, 1980), blue berries (El-Agamy *et al.*, 1982), *Phaseolus* (Rabakoarihanta *et al.*, 1979; Shii *et al.*, 1982) and *Crocus sativus* (Chichiricco and Caida, 1984).

In self-and cross-pollinations on *Sesamum* pollen grains germinate within one hour and penetrate the stigmatic surface. Many of the tubes reach half of the stylar length within 6 hrs and by about 12 hrs, the upper part of the ovary. Inhibition occurs in the style between 10-12 hrs when tubes burst at different levels of the style (present investigation) indicating that not all the tubes follow the same rate of growth. The ungerminated grains indicate inhibition at stigmatic level. Incompatible tubes grow very slow and they cannot reach the ovary and are inhibited at different levels. Only compatible tubes grow very fast and reach the top of the ovary before the critical inhibition period. Ovule penetration by 12 hrs after pollination in *S. alatum* x *S.orientale* supports this. The pollen tubes penetrate the ovules near the stylar end first (present investigation). Dadlani (1958) reported that the pollen tubes reach the
ovules at the top, middle and bottom regions in the ovary within 12, 24 and 36 hrs respectively in the cross *S. occidentale* x *S. prostratum*.

In apple (Modlibowska, 1945) and *Eucalyptus* (Ellis et al., 1991) the upper part of the style is the major part of arrest of interspecific pollen tube. Sears (1937) in *Nimesia strumosa* and Atwood (1941) in *Trifolium repens* reported that pollen grains stop at the top of the ovary. Similar results are observed in the interspecific crosses of *Panicum antidotale*, *P. coloratum* and *P.deustum*, where many of the pollen tubes are arrested at stigma, style and very few reach the ovary but become disoriented and never penetrate the ovule (Burson et al., 1983). But in *Sesamum* inhibition occurs on the stigma and all along the entire length of the style (present investigation).

Evans (1962) Chen and Gibson (1972) reported, in the cross of *Trifolium repens* with its relatives, pre-fertilization barriers are not the only cause for cross-incompatibility and fertilization occurs in all crosses with low frequency. Similarly in the present investigation, stigma and style are not the barriers to interspecific pollen germination and tube growth because normal pollen tubes are also found entering the ovules.

Pollen, stigma and stylar treatments can be employed only when there is complete absence of pollen germination or tube inhibition at any particular site. Sometimes stigma and pollen treatments with organic solvents have no effect. In *Lilium*, interspecific incompatibility could not be overcome by hot water treatment (Ascher and Peloquin, 1968). Stettler et al., (1980) found no effect of hexane treatment, GA, NAA of stigma and pollen in poplars. Visser (1981) reported that dead methylated pollen does not improve seed set after self and cross pollinations in apple and pear. Roggen et al., (1988) reported in *Lilium* interspecific crosses after cut-style method many pollen tubes stop in front of the micropyle. In the present investigations on *Sesamum* pollen germination and inhibition are seen in one and the same pistil. Moreover treatments of stigma with methanol completely inhibited pollen germination in artificial self-pollination of *S.orientale*, *S.alatum*, *S.laciniatum*
and *S. radiatum* (personal observation). This may be due to the removal of stigmatic substances which are essential for pollen stigma interaction. Sastri & Shivanna (1976) observed complete absence of germination of *S. mulayanum* pollen on the stigmas of *S. indicum*. But methanol-treated self pollen grains induced other non-treated grains to germinate which however fail to cross the style and they concluded incompatibility at the stylar level. But, Mitra and Biswas (1992) are able to get good seed set and hybrids in the same cross without any treatment. Riccharia (1934) reported germination of *Martynia diandra* pollen on the stigmas of *S. indicum*. The Results stated hereabove strongly support that stigma and style are not the barriers in *Sesamum*.

According to Hamilton (1976) compatible pollen contains specific enzymes which dissolve the waxy coating of the stigmatic surface, thus coming in contact with the growth promoting substance of the stigma they germinate, whereas incompatible pollen lack these enzymes and cannot do so and hence fail to germinate. This is consistent with our present investigation.

It is worth mentioning that in *Sesamum* (present investigation) abnormalities are not observed in the ovary outside the ovule. Moreover the pollen tubes which enter the ovary grow normally along the placental tissues and enter through the micropyle. Kho and Bääer (1970) observed that difficulties occur in the ovary before pollen tube entry in *Rhododendron impeditum* x *R. williamsianum* the mechanism of which is not known.

Patel and Datta (1960) reported in *Corchorus olitorius* x *C. capsularis*, the growth of the pollen tubes is very slow and fertilize the ovules in the upper region of the ovary. Marshal and Ellstrand (1986) reported that in *Raphanus*, the pollen tubes growing at slow rate fertilize the ovule situated at the stylar end whereas those with faster growth fertilize ovules at the basal portion of the ovary. Meinke (1982) also reported similar results in *Arabidopsis* whereas in *Sesamum* (present investigation) such clearcut sequence is not observed. The pollen tubes fertilize ovules in a random manner. Moreover successful pollen tube penetration into the ovule is not
accompanied by healthy seed set. But it ensures pod development. Microscopic studies suggest that if pollen tube enters at least one of the ovules pod development proceeds normally irrespective of post-fertilization barriers. Number of healthy seeds indicates successful fertilization leading to the embryo and development of the endosperm, the shrivelled seeds indicate the number of ovules in which post-fertilization degeneration occurred and the degenerated ovules suggest where there is no pollen tube entry or no fertilization. In the crosses and reciprocal crosses if pollen tubes do not enter into the ovary, they wither off 3 or 4 days after pollination and their percentage indicate the extent of pre-fertilization barriers which is 100% in *S. orientale* x *S. alatum* cross.

In the crosses of *Trifolium*, Chen and Gibson (1972) reported highest frequency of fertilized ovules in which growth of the pollen tube is very similar to that of the control and the lowest frequency in the combination in which the tube growth deviated to the maximum from control. Such a correlation is not possible in *Sesamum* because there is 100 seed and pod set in the parents while it is highly reduced in crosses and the pollen tube behaviour is similar in all self, cross pollinations. Many ovules with pollen tubes are found in collapsed state without fertilization (present investigation). Similar results are observed in the crosses of *Cucumis* (Kho et al., 1980) due to post-fertilization barriers, but parthenocarpic fruits are obtained.

In the present investigation fertilization is completed within 24 hrs in all the crosses except in *S. laciniatum* x *S. orientale* where it is extended by another few hours. Further development of the embryo is delayed depending upon the nature of species used as female parent. In *S. orientale* x *S. laciniatum* and *S. orientale* x *S. radiatum* the development of zygote is delayed up to 5 days. In the reciprocal crosses, the divisions of the zygote is after 2 days in *S. alatum* x *S. orientale* and in *S. laciniatum* x *S. orientale*, *S. radiatum* x *S. orientale* it is after 8-9 days. In the compatible ovules the development of the embryo follows the same pattern as that of the parents whereas in the incompatible ovule endosperm degenerates within 72 hrs
which is too early for the zygote to divide. Hence further development of embryo is not observed in these ovules and they develop into shrivelled sterile seeds.

Various interpretations have been offered by several authors for the failure of embryo and endosperm in interspecific crosses. Kihara and Nishiyama (1932) reported in the interspecific crosses of *Avena strigosa* (*n* = 7) x *A. fatua* (*n* = 21) less retarded development of endosperm and absence of mitotic irregularities when the female parent has the higher chromosome number, but the rate of development is slowed down and speeded up in low x high cross. According to them, an excessive number of chromosomes brought in by the male gamete in low x high crosses exerts strong stimulus on the endosperm nuclei and speeds up the growth. Wakakua (1934) reported in *Triticum* crosses when higher chromosome number is used as female, seed set is poor but endosperm development is more rapid than in the mother and still more in reciprocal direction. He suggested genome imbalance between male nucleus and the polar nucleus on the one hand and the male nucleus and the egg on the other hand. Boyes and Thompson (1937) also suggested chromosome imbalance for endosperm failure.

Brink and Cooper (1941) assumed that normal seed development is considered to be contingent upon a certain balance between reaction norms of these genotypically diverse tissues and the genetic make up of the endosperm is quantitatively much more like that of the associated maternal tissues than the embryo. They also assumed that early post fertilization behaviour is highly critical and if the primary morphogenetic changes occurring at this stage fail, the secondary mechanism for nourishing the embryo is concurrently determined.

Stephens (1942) suggested that the extent of differences in 'genetic strengths' between the species having different chromosome numbers would account for the success or failure of the cross. Thompson and Johnston (1945) attribute the endosperm failure in *Hordeum vulgare* x *Secale cereale* to the endosperm constitution itself or to the reaction between the female parent and the hybrid endosperm because genic conditions are not the same in the endosperm as in the embryo.
Hakansson and Ellerström (1950) interpreted the disturbed tissue relations for the seed failure in the crosses between diploid and tetraploid rye.

Beaudry (1951) reported in the cross *Elymus virginicus* x *Agropyron repens* that the sperms of *A. repens* modify the physiology of the antipodals of *E. virginicus* which alter the nutrient supply to the endosperm leading to the disorganisation of the tissue. Greenshields (1954) suggested genetic difference between the endosperm and maternal tissues as the cause for the endosperm failure. In the reciprocal crosses the genetic constitution of the zygote is the same, but the endosperm constitution is different, with the female parent supplying two genomes to one from the male parent. Brock (1954, 1955) reported that chromosome breakage during mitosis leads to degeneration of endosperm in *Lilium* and in garden hyacinths.

According to Weaver (1957) physiological imbalance between the embryo and the endosperm produced by the embryo cause endosperm degeneration in cotton. Reusch (1959) postulated that the anomalous behaviour of the endosperm in the hybrid seed is due to disturbances in the nucleic acid metabolism, which in turn is a consequence of an unfavourable nuclear-cytoplasmic relationship.

Gill and Waines (1978) reported that a pollen factor which shows dosage effect interact with the maternal genome leading to endosperm abortion. Johnston *et al.*, (1980) proposed Endosperm Balance Number (EBN) which explains that the endosperm develops abnormally in the interspecific crosses when the maternal : paternal genome ratio deviates from 2:1 in the endosperm itself.

Lin (1984) interpreted that a seed consists of 3 different components i.e., maternal tissue, endosperm and embryo. Endosperm failure may be due to differences in the polidy level of the above components. It may be because of 1) deviation from 2:3:2 ratio of maternal:endosperm:embryo (Muntzing, 1933); 2) deviation from a 3:2 ratio of endosperm:embryo (Watkin 1932); 3) deviation from 2:3 ratio of maternal: endosperm (Valentine, 1954). Charnov (1979), Bawa and Webb (1984) and Lloyd (1987) suggested maternal regulation of offspring quality as a
primary cause of seed abortion whereas Wiens et al. (1987) suggested genetic load as the major factor.

All the above explanations are applicable for the failure of endosperm in incompatible ovules of Sesamum hybrids. But it is impossible to consider a ploidy level interpretation since the chromosome numbers of S. orientale, S. alatum, are not exact multiple of the basic number. It is also difficult to interpret the formation of healthy endosperm and embryo in compatible ovules of the same pod (Present investigation). It is interpreted that in Sesamum post-fertilization barriers also occur. If the male gametes are matching, development proceeds in a normal way otherwise that combination results in failure through endosperm degeneration.

Degeneration of endosperm is accompanied by persistence of endothelial layer along with a few adjacent layers, which otherwise degenerate in the parents and in the compatible ovules (present investigation). This can be compared to the proliferation of the maternal tissues which is referred as 'somatoplastic sterility' by Cooper and Brink (1940). They suggested in Medicago sativa, Nicotiana and Lycopersicon the abnormal tissue adjacent to the embryo sac has interrupted the translocation between endosperm and the vascular tissues. In the interspecific crosses of Datura, the multiplication of cells of the endothelium results in the formation of tumor which absorbs the endosperm and embryo (Satina et al., 1950). In the interspecific crosses of Arachis hypogaea × A. diogoi (Johansen and Smith, 1956) as well as in the present investigation, endosperm collapse is followed by hyperplastic development of endothelial layer. The ovules, thus affected, develop into empty seeds.

In the compatible ovules, endosperm and embryo follow normal pattern of development and the histochemical localization of RNA, protein and insoluble polysaccharides reveal similar pattern as that of their parents. Interspecific barriers are observed before and after fertilization. The extent of failure is more with low seed set (present investigation).
The literature on the interspecific hybridization in *Sasamum* is extensive. Final results in the present study on pod, seed set, interspecific hybrid and amphidiploid of *S. orientale* x *S. laciniatum* coincide with the previous reports mentioned in the review. All the workers have concentrated on final pod set, cytology of the hybrid, amphidiploid and sesquiploids of *S. orientale* x *S. laciniatum*. Dhawan (1946) reported successful seed set in the cross, *S. radiatum* x *S. orientale* as in the present investigation. Dadlani (1958) had found post-fertilization abortion of the embryo and endosperm in *S. occidentale* x *S. indicum* and *S. occidentale* x *S. prostratum* respectively. No fruit set occurs in the reciprocal cross. They did not mention the pollen tube and other abnormalities during pre-, and post-fertilization and they interpreted that the crosses are successful since the female parent has higher chromosome number and the seed failure is due to difference in chromosome number. Subramaniam (1972) has assumed pre-, and post fertilization barriers in the crosses *S. indicum* x *S. laciniatum*, *S. laciniatum* x *S. occidentale* and interpreted that failure may be due to structural difference in the chromosome of species having same chromosome number and in other cases it is attributed to the difference in chromosome number.

The cross *S. orientale* x *S. alatum* is a complete failure including present investigation. Both the species have same chromosome number and only pre-fertilization barriers are observed and there is no pollen tube entry except in very few ovules. Abcisson of the pods takes place within 3 or 4 days after pollination. Hence pre-fertilization barriers are the exclusive cause for the failure. But the reciprocal cross yields 0.4% pod set. Pollen germination, development of the embryo and endosperm and degeneration are the same as in other crosses. The above results strongly indicate unilateral incompatibility that exists between the two species (present investigation). Similar results are obtained in the cross *Vigna anguiculata* (2n = 22) x *V. vexillata* (2n = 22) where the cross fails within 24 hrs or 48 hrs after pollination but results in hybrids if *V. vexillata* is used as the female parent (Barone et al., 1992). Brink and Cooper (1947) and Greenshields (1954) suggest that failure
results from combining qualitatively different genomes. Both schools do not specify the type of incompatibility among the species. Thompson (1930b) assumed that although the chromosome numbers are same, the cytoplasm of one parent lacks the proper materials to enable certain genes of the other to produce their normal characters. Lewis and Crowe (1958) suggested antigen-antibody reaction for the unilateral incompatibility between self-compatible species.

In the present investigation, the cross *S. orientale* x *S. laciniatum* results in interspecific hybrid as reported by Ramanathan (1950), Aiyadurai *et al.*, (1962), Subramaniam and Chandrasekaran (1977). The morphological characters of the present hybrid and its amphidiploid are similar as reported by these authors. But it is observed in contrast to the previous reports both hybrid and amphidiploid are susceptible to phyllody.

The cross *S. orientale* x *S. radiatum* and its reciprocal cross result in the formation of healthy and sterile seeds. But the seeds either fail to germinate or if they germinate the seedlings die off at early stage (present investigation). Garu (1934) and Mazzani (1952) obtained inviable seeds in the same cross.

Present embryological studies on the hybrid and amphidiploid of *S. orientale* x *S. laciniatum* reveal similar developmental patterns as that of the parents. Stamens consist of degenerated and healthy anthers. In the degenerated anthers early degeneration of sporogenous cells before tapetal differentiation or degeneration of both tapetum and sporogenous cells at pre-meiotic stage are observed. In the healthy anthers development proceeds normally. But resulting tetrads show abnormalities such as polyads, microspores of different sizes, hypertrophy or abnormal divisions of the pollen nucleus in the amphidiploid. Low quantity of Ubisch granules are produced. Sterility may be due to the meiotic irregularities as reported in the hybrids by Ramanathan (1950), Aiyadurai *et al.*, (1962), Subramaniam and Chandrasekaran (1977). Ramanujam (1944) reported high pollen sterility in spite of regular meiosis in the amphidiploid of *S. orientale* x *S. prostratum*. Further, development of empty
seeds may be due to genic effect on the pollen and not on ovule development. He suggested high female fertility in the amphidiploid.

Pre-meiotic and post-meiotic degeneration of tapetum and sporogenous tissues are characteristic features of male-sterile anthers (Chauhan, 1979). Lunyeva et al., (1970) stated that due to meiotic irregularities the pollen grains of the F₁ are deficient of cytochrome oxidase and hence show maximum sterility. Brigette and Sievers (1981) reported that fertile pollen is produced only if correct development of the tapetum is coordinated in time with spore development. According to Panchaksharppa and Annigeri (1984) anther sterility is due to the deficiency of carbohydrate source from the maternal parent which in turn affect the callose wall around pollen mother cells, tapetal function, endothelial thickenings and pollen wall formation leading to sterility. Chauhan (1979) stated that formation of endothelial thickenings is regulated by the tapetal cells and lack of endothelial development is associated with male-sterility and in these anthers, tapetal layers fail to degenerate or the cells become hypertrophied during post-meiotic stages. In contrast to this report, in the present investigation, normal tapetal behaviour is observed followed by the formation of fibrous thickenings of the endothecium as in Capsicum (Horner and Rogers, 1974) and Helianthus annuus (Hegde and Isaacs, 1992). Hence the sterility of the microspores is attributed to the difference in chromosome number and the disharmony between the parents.

Present investigation on ovule development in hybrid and amphidiploid shows weak megaspore mother cell formation and its subsequent degeneration either before or after meiosis. But as the integument develops normally the ovules are represented by integument only. Normal embryo sac also develops following the same pattern as that of the parents. It is observed that there is no seed due to high pollen and ovule sterility. Back cross with both the parents is also a failure due to ovule sterility. Similar type of ovule sterility is observed in the F₁ hybrid of Nicotiana (Green leaf, 1941), red clover (Povilaitis and Boyes, 1957), Solanum melongena x S. macrocarpon (Rajasekaran, 1970; Gowda et al., 1990). Dobzhansky (1951)

Pollen grains show 50% viability in the amphidiploid and few ovules are fertile with well formed embryo sac (present investigation). Stebbins (1950) suggested that in hybrids and in amphidiploids degeneration at early stages of development is due to genic disharmony and the male reproductive organs are easily affected than the female. According to him failure of hybrids is due to structural differences in the chromosomes of the parents and in the amphidiploid it is due to non-synchronization of the parental chromosomes in the meiotic cycle. But partial fertility of the amphidiploid of *S. orientale* × *S. laciniatum* may be due to the presence of harmony of the chromosomes to a certain extent and its doubled nature.

In vivo pollination studies on the back cross of the hybrid fails due to high stylar inhibition and ovule sterility. Random pollen tube growth in the ovary may be due to lack of any chemotropic substance at the micropyle of the sterile ovule for attracting the pollen tube.

In the amphidiploid pollen germination inhibition at stigma and style resemble that of the parents. Only viable pollen grains germinate. Due to ovule and embryo sac sterility, fertilization occurs only in very few ovules. Post-fertilization degeneration of endosperm and zygote results in empty seeds. Subramaniam and Chandrasekaran (1977) reported that the sterile seeds of the amphidiploid of *S. orientale* × *S. laciniatum* contain thin endosperm. In the present investigation it is observed that the sterile seeds contain only the seed coat with persisting endothelium along with a few layers of the integument enclosing empty cavity. Healthy seeds contain normal embryo.

From the foregoing results the following points are clear.

1) The species of *Sesamum* follow same developmental and histochemical patterns in anther, ovule, embryo and endosperm.
2) The cultivated and wild species of *Sesamum* ie., *S. orientale* and *S. alatum* respectively which have similar chromosome numbers show self-incompatibility at pre-fertilization level.

3) The cross *S. orientale* x *S. alatum* is a complete failure and the reciprocal cross results in low seed set and the fertile hybrid obtained by Ramalingam *et al.*, (1992) suggest unilateral incompatibility among the two species.

4) The other two species *S. laciniatum* and *S. radiatum* differ widely in their chromosome numbers. Pre-fertilization inhibition of pollen tubes, less number and degeneration of ovules result in low seed set in *S. laciniatum*; weak endosperm formation results in the formation of brown sterile seeds in *S. radiatum*. Their crosses and reciprocal crosses with *S. orientale* show inhibition at pre- and post-fertilization levels and low percentage of healthy and sterile seeds. This indicates bilateral incompatibility.

5) Irrespective of the direction of the cross the abnormalities are the same both at pre- and post-fertilization stages. According to Stebbins (1942) interspecific incompatibility is genetically determined and partial success is common in related species.

6) Both male and female sterility are the reasons for the complete sterility of the hybrid and the amphidiploid shows partial sterility.

In any interspecific cross, to achieve successful fertilization and seed set it is essential that all the pistil characters of the female parent should accurately interact with the pollen tube and male gamete of the male partner. Based on this, Hogenboom (1975, 1984) formulated two mechanisms for non-functioning of gametes in interspecific crosses: i) incompatibility which prevents functioning of both parents and ii) incompleteness of relationship due to lack of genetic information in one partner about some moment between pollination and fertilization the barrier operates and inhibits pollen tube growth. The barrier may operate even at post-fertilization level resulting in embryo abortion, hybrid weakness and hybrid sterility.
Hogenboom (1975) proposed the term incompatibility for the former and incongruity for the latter phenomenon.

In the present investigation on interspecific crosses among the species of *Sesamum* all types of abnormalities such as inhibition of pollen germination, non-directional growth of the pollen tube, pollen tube burst at different levels of the style, lack of fertilization, zygote and endosperm degeneration, persistence of endothelium are observed. Together normal pollen germination, tube growth, fertilization, embryo and endosperm development are also observed in one and the same ovary. It is interpreted that the pollen and pistil characters are matching there will be normal development, otherwise inhibition occurs depending upon the level at which the barrier operates. It is evident that there must be multiple crossing barriers among the species of *Sesamum* which make the system more complicated. This deviates from normal incompatibility systems such as sporophytic where there is stylar inhibition which can be easily broken by various treatments. Present investigations on *Sesamum* poses such a complicated situation indicating high degree of incongruity among the species.
Seed morphology

Seeds of Sesamum are morphologically classified based on their colour- black or white, on texture- smooth or rugose, on the size- small, medium and large (Kashi Ram, 1930; Joshi, 1961). In the present investigation, it is found that the seeds of S. orientale, S. alatum, S. laciniatum and S. radiatum are uniformly medium sized. But the shape varies from oval (S. orientale, S. laciniatum) to triangle (S. alatum, S. radiatum). Even though the number of seeds per pod are less in S. laciniatum the 1000 seed weight is 2.4681 gms which is almost near to 3.1892 gms of S. orientale. In S. alatum and S. radiatum number of seeds are more but their weight is much less. The weak embryos of brown seeds of S. radiatum, winged nature of S. alatum may be the reasons for this low weight and bulkness of the seeds in the other two species for their heaviness.

The structure of the integument at the time of fertilization has been described elsewhere. Singh (1960, 1963 and 1964) and Vaughan (1970) reported that the seed coat consists of a few layers of the integument along with the epidermis. Whereas in the present investigation it is observed that the seed coat is derived from the outermost layer of the integument alone. The seed coat of S. orientale, S. alatum, S. laciniatum and S. radiatum follow the same type of development (present investigation). The development proceeds up to the globular stage of the embryo. Longitudinal section of the seed coat shows that the cells are radially elongated and completely separated from the underlying layers. The surface of the seed coat appears uniform in S. orientale but wavy in other species i.e., S. alatum, S. laciniatum and S. radiatum, due to unequal radial elongation. Deposition of wall materials are less and hence the seed coat is smooth, thin and soft in the cultivated S. orientale thus facilitating germination. But hard seed coat prevents inhibition of water and hence seed germination is slow in the wild species.

In spite of the structure the seed coat has such an impact on storage and germination. Its surface has not been studied except for the report of Vaughan (1970) and Pandey and Dogra (1992). In the present investigation the seed surface
helps in distinguishing the species. Smooth seeds of *S. orientale* stands unique among the species of *Sesamum*. The other species *S. alatum*, *S. mulayanum*, *S. laciniatum*, *S. prostratum*, *S. radiatum* have ridges on the surface and hence the seed coat is rough. Heavy deposition of wall materials and well elevated ridges form thick reticulate pattern in *S. alatum*. But the ridges are not so thick in other species. In *S. laciniatum*, the ridges are across the longitudinal axis whereas in *S. radiatum* they run only along the periphery. The ridges reveal reticulate pattern in *S. mulayanum* and *S. prostratum*.

Light microscopic studies show that the individual cells are isodiametric in all the species but reveal that they differ among themselves. SEM studies on the surface of the seeds of *S. indicumis* reported to have tuberculate cells with globular protuberance which coincided with our present investigation where the individual cells have convex upper surface in *S. orientale* and *S. alatum*. But in all the remaining species the cells are concave. The end walls are even in *S. mulayanum* and uneven in *S. prostratum* and *S. radiatum*. But end walls of *S. laciniatum* are inconspicuous. These characters are much useful in the identification of the species in *Sesamum*.

Based on the surface features an identified key is cation given:

Seed coat smooth without any ornamentation ............................................. *S. orientale*

Seed coat rough

Ridges are reticulate, cells have concave faces, ridges form deep pits ........................................................................................................ *S. alatum*

Cells have concave faces ridges form shallow pits

Cells are smooth with irregular end walls ........................................... *S. prostratum*

Cells are thick with even end walls ............................................ *S. mulayanum*

Ridges run across the longitudinal axis of the seed .......................................................... *S. laciniatum*

Ridges are seen only along the periphery .................................................................... *S. radiatum*