Chapter-V

DISCUSSION
DISCUSSION

Among the higher plants, the members of Asclepiadaceae and Orchidaceae have evolved a fascinating array of unique features during microsporogenesis and gametogenesis (Galil and Zeroni, 1969; Vijayaraghavan and Shukla, 1976, 1977; Vijayaraghavan and Cheema, 1978; Sreedevi and Namboodiri, 1982; Sood, 1986; Vijayaraghavan et al., 1986; Viswanathan and Lakshamanan, 1986; Vijayaraghavan, Gupta and Shukla, 1987; Vijayaraghavan et al., 1987; Hegde et al., 2000). In most of the members of these families microspores in a pollen sac remain in aggregation to form a single mass the pollinium. The pollinium is transferred in toto flower to flower by the pollinating insects.

During last three decades considerable histochemical information is available on anthers, which produce pollen grains in monads (Heslop-Harrison, 1972; Mascarenhas, 1975; Bhandari and Sharma, 1983; Blackman and Yeung, 1983; Panchaksharappa et al., 1985; Shivanna and Johri, 1985; Noher de Hallac, 1990; Sheel and Bhandari, 1990; Hegde et al., 1993; Vijayaraghavan and
But, despite the unique morphological and anatomical features of pollinia, it is disappointing to note that histochemical investigation on the pollinial development is limited to only a handful of reports (Vijayaraghavan and Shukla, 1976, 1977; Vijayaraghavan and Cheema, 1978; Vijayaraghavan et al., 1986; Vijayaraghavan, Gupta and Shukla, 1987; Vijayaraghavan et al., 1987; Hegde et al., 2000). Hence, in the present thesis comparative histochemical study on certain aspects of anther differentiation in Moringa oleifera Lam. (Moringaceae), Dregea volubilis (L.f.) Benth. ex Hk.f. (Asclepiadaceae) and Dendrobium ovatum (Willd.) Kranz. (Orchidaceae) is carried out to ascertain similarities and variations associated with pollen development in the anthers that produce monad and polyad types of pollen grains.

The histochemical, ultrastructural and molecular studies on anthers reveal that the development, differentiation and function of anther tissues are genetically controlled and pollen formation involves the coordinated functioning of both diploid and haploid gametophytic tissues (Kaul, 1988; Koltunow et al., 1990; Bedinger, 1992).
Anther development is divided into two phases (Goldberg et al., 1993). During phase-I, the establishment of anther morphology, cell/tissue differentiation and meiosis in meiocytes occur. During phase-II, differentiation of pollen grains, enlargement of anther, tissue degeneration, dehiscence and pollen release occur.

The overall pattern of development of anther is not different in *M. oleifera*, *D. volubilis* and *D. ovatum*. The epidermis, endothecium, middle layer(s) and tapetum enclose the fluid-filled locule. The locule contains the sporogenous cells/meiocytes that will undergo meiosis, producing tetrads. The microspores undergo asymmetric mitotic division to produce bicelled pollen. During post-meiotic stages, tapetum degenerates while the endothecium undergoes structural elaboration.

Yet, the details of microsporogenesis, presented below, in these three plants differ significantly from one another. The deviations include both structural and histochemical features. The following discussion is an attempt to analyze how the unique
developmental events, observed in the anthers that produce polyads, play a vital role in the formation of pollinium.

PREMEIOTIC PHASE:

Early stages of microsporogenesis in all three plants studied in the present investigation are identical. The archespore cells are densely cytoplasmic and rich in RNA and total proteins (Vijayaraghavan, Gupta and Shukla, 1987; present study). Transitory accumulation of PAS-positive granules is reported in Pergularia daemia (Vijayaraghavan and Shukla, 1977) and Calotropis procera (Vijayaraghavan and Cheema, 1978).

The primary sporogenous and primary parietal cells, produced by the periclinal division of archespore cell, are featured by dense cytoplasmic RNA and proteins, in addition to cytoplasmic polysaccharides in D. volubilis (present study). Primary sporogenous cell divides in various planes to produce mass of compactly arranged sporogenous cells (Freudenstein and Rasmussen, 1996; present study). Sporogenous cells are rich in RNA, proteins and cytoplasmic polysaccharides (present study). Free ribosomes
present in the sporogenous cells are responsible for dense basophilic cytoplasm of these cells (see Hegde et al., 1993). In *Citrus* sporogenous cells possess lipids (Rudramuniyappa and Hegde, 1985) and in *Datura* (Hegde and Andrade, 1982; Andrade and Hegde, 1983) and *Helianthus annuus* (Hegde and Isaacs, 1992) sporogenous cells show peak activities of non-specific esterases, glucose-6 phosphatase, alkaline and acid phosphatase, peroxidase, malate dehydrogenase, succinic dehydrogenase and cytochrome oxidase. These indicate that sporogenous cells are equipped with metabolic machinery needed for their growth and differentiation.

Presence of rich cytoplasmic polysaccharides in the sporogenous cells of all the three plants studied in the present investigation is a rare feature. Occurrence of starch storage in the sporogenous cells is reported in *Euphorbia* (Rudramuniyappa and Annigeri, 1985), *Calanthe* (Hegde and Rudramuniyappa, 1986) and *Spathoglottis* (Hegde et al., 2000). As opined by Vijayaraghavan and Cheema (1978) and Vijayaraghavan and Sudesh (1994), the storage carbohydrates of sporogenous cells serve as energy source for metabolic activities needed for the differentiation of meiocytes.
A correlation between the enlargement of sporogenous cells, prior to and during the differentiation of meiocytes and reduction in the cytoplasmic polysaccharides in, *M. oliefera*, *D. volubilis* and *D. ovatum* provide circumstantial evidence in support of this contention (present study).

Like any other tissue that undergoes growth and differentiation, the sporogenous and tapetal cells are also thin walled with negligible deposition of cellulose. This feature is also observed in the sporogenous cells of *M. oliefera* and *D. ovatum* (present study). However, the sporogenous cells and tapetum of *D. volubilis* are exceptional in possessing fairly thick primary walls, rich in general polysaccharides (present study). Interestingly, these cell walls lack noticeable deposition of cellulose. A definite correlation is observed between the synthesis of distinct PAS-positive cell walls and distributional pattern of ascorbic acid (present study). The sporogenous cells and tapetum of *M. oliefera* and *D. ovatum*, which lack distinct PAS-positive and cellulosic wall, are devoid of ascorbic acid whereas these anther cell types in *D. volubilis* possess ascorbic acid along their primary wall.
These observations suggest that the presence of ascorbic acid in the sporogenous cells and tapetum of *D. volubilis* might be associated with the development of the distinct primary wall, in particular, and various other metabolic processes in these anther-cell types. The development of relatively thick wall in the sporogenous and tapetal cells of *D. volubilis* seems to have a far-reaching significance in the development of pollinium, which will be discussed later.

The last cell generation of sporogenous tissue differentiates into meiocytes. Not much histochemical and ultrastructural variation, except the synthesis of ascorbic acid along the meiocyte walls in *D. ovatum*, are observed between young meiocytes and sporogenous cells (Vijayaraghavan and Cheema, 1978; Vijayaraghavan and Sudesh, 1994; present study). Active mitochondria biogenesis in the meiocytes of *Solanum nigrum* (Bhandari and Sharma, 1988), *Carica papaya* (Sheel and Bhandari, 1990) and *Helianthus annuus* (Smart *et al.*, 1994) reflect the metabolic potential of these cells. Rich RNA and protein synthesis during pre-meiotic stages is thought to be essential for structural organization of chromosomes (Vijayaraghavan and Cheema, 1978).
The deposition of callose, a β 1-3 linked glucose polymer, around the meiocytes is a phenomenon of almost universal occurrence in angiosperm anthers, including *M. oliefera* (present study). There is a switch over in glucan synthesis from β 1-4 glucan (cellulose) to β 1-3 glucan (callose). Such switch over in glucan synthesis is induced by the alteration in calcium ion concentration at the plasma membrane (Worrall *et al.*, 1992). According to Kauss (1987) high cytoplasmic Ca++ content activates the β 1-3 glucan synthase present on the cell membranes. Callose synthesis continues until the formation of primexine around microspores (Blackmore and Barnes, 1988). In many plants callose is PAS-positive (Bhandari and Sharma, 1983; Katti *et al.*, 1994; Vijayaraghavan and Sudesh, 1994). *M. oliefera* resembles *Cannabis sativa* (Heslop-Harrison, 1964), *Chenopodium rubrum* (De Fossard, 1969) and *Iphigenia pallida* (Panchaksharappa and Syamasundar, 1974) in having PAS-negative callose. Such histochemical variation may account for heterogeneous chemical nature of callose (El-Ghazaly and Jensen, 1987). Important morphogenetic significance has been attributed to the temporal and spatial occurrence of callose during anther development.
According to Heslop-Harrison and Mackenzie (1967), Knox and Heslop-Harrison (1970) and Southworth (1971) callose prevents the transport of high molecular weight substances and thus functions as a ‘molecular filter’ insuring genetic autonomy during transition from diploid to haploid phase (Carafa and Pizzolongo, 1990). Because of this feature of callose, meiocytes acquire chemical properties different from the surrounding sporophytic tissues, which perhaps is a prerequisite condition for meiosis to occur (Heslop-Harrison, 1972; Stanley and Linskens, 1974; Steiglitz, 1977; Shivanna and Johri, 1985). Due to its impermeable nature, callose isolates the meiocytes and their meiotic products from the surrounding sporophytic tissues as well as prevents cell cohesion and fusion (Waterkeyn, 1962; Heslop-Harrison and Mackenzie, 1967; El-Ghazaly and Jensen, 1987; Theis and Robbelen, 1990; Bedinger, 1992; Bhandari and Khosla, 1995). According to El-Ghazaly and Jensen (1987) the central callose helps to keep microspores pressed against the walls of the tapetal cells. Barskaya and Balina (1971) are of the opinion that callose prevents premature swelling of microspores and provides protection to meiocytes from dehydration. Also, callose has been
attributed with the function of maintenance of enzymes associated with primexine and exine formation in proper functional conditions and prevention of random oxidation and auto-polymerization of sporopollenin precursors (Vijayaraghavan and Shukla, 1977). According to Blackmore and Barnes (1988) callose provides the cytoskeleton needed to establish microspore polarity. Absence or poor deposition of callose or its untimely dissolution resulting in the formation of non-functional pollen grains supports the roles attributed to the callosic wall (Hegde and Isaacs, 1992).

However, because of the localization of cerium peroxide, a highly electron dense material, within the callose surrounding microspore tetrads and on the primexine of microspores of *Beta vulgaris*, Rodriguez-Garcia and Majewska-Sawka (1992) are of the opinion that callosic wall of meiocytes is not an impermeable barrier. Further, occurrence of normal meiosis and cytokinesis in the absence of callose have been reported in *Pergularia daemia* (Vijayaraghavan and Shukla, 1977), several submarine hydrophilous flowering plants (Ducker *et al.*, 1978; Pettitt, 1981), transgenic tobacco (Worrall *et al.*, 1992) *Ceratophyllum* (Takahashi,
1995) and *Spathoglottis* (Hegde *et al.*, 2000). In the present study also normal meiosis is observed in the callose-less meiocytes of *D. volubilis* and *D. ovatum*. These results are not in support of the contention that initiation of meiosis is associated with the presence of callose as opined by Heslop-Harrison (1972), Stanley and Linskens (1974), Steiglitz (1977) and Shivanna and Johri (1985). According to Delmer (1987) callose is not a part of the cell wall and according to Worrall *et al.* (1992), because of absence of clear evidence of its essential role, anther callose is to be considered as an accidental, non-functional product of callose synthase activity induced by increased calcium ion concentrations associated with other cellular processes. If so, it may be suggested that, *D. ovatum* and *D. volubilis* have evolved a mechanism for the partial and complete elimination of the synthesis of ‘accidental’ callose respectively.

If anther callose is ‘accidental’ and non-functional, then it is intriguing why it makes its appearance and disappearance at precise stages of anther development in most of the angiosperm plants? The absence or poor synthesis of callose and its untimely
dissolution resulting in the formation of sterile pollen point out its vital significance in the other processes related to production of fertile pollen, if not in the induction of meiosis. One such function of callose is envisaged by van Amstel and Kengen (1996). These authors implicate that callose functions as a temporal stabilizer of the newly incorporated plasmamembrane by regulating the direction of cellulose microfibrils deposition.

The architecture of the anthers of D. volubilis and D. ovatum suggests that isolation of meiocytes from the surrounding sporophytic tissue is an indispensable provision required for the induction of normal meiosis. In D. volubilis, at meiocyte stage, an acellular deposition, rich in general polysaccharides, but not in cellulose, appears on the inner tangential face of the tapetum. Although speculative, this layer, being located between the meiocytes and tapetum, effectively isolates the meiocytes from the influence emanating from the surrounding sporophytic tissues. At later stages of anther, tapetally derived pollinial coat becomes ascorbic acid-rich, and at maturity it contains sporopollenin.
In addition to tapetally-secreted layer, the thick PAS-positive primary wall of meiocytes may also provide some degree of isolation to individual meiocytes. The persistent PAS-positive wall of meiocytes during meiosis may pose some disadvantage by blocking the transport of metabolites, to some extent, but the meiocytes overcome this short-coming as they are metabolically sustainable due to the storage cytoplasmic polysaccharides, RNA, total proteins and ascorbic acid (present study). The reduction in the cytoplasmic polysaccharides in the dyads provides circumstantial evidence in support of this contention.

In *D. ovatum*, prior to meiosis, a thick deposition of acellular layer occurs between the tapetum and meiocytes. This deposition is autofluorescent and rich in general polysaccharides, cellulose, ascorbic acid and proteins. This layer possibly originates in the tapetal cells because the cytoplasm of the tapetal cells contains similar substance. Later, during meiosis, this wall material deposits on the outer face of peripherally located meiocytes. After meiosis, this substance penetrates into the locule. In addition to this layer, callose deposition occurs on the outer face of peripheral meiocytes.
Thus, the deposition of callosic wall and tapetally secreted acellular deposition present between meiocytes and tapetum provides isolation to the entire mass of meiocytes. In both *D. volubilis* and *D. ovatum*, the absence of callose around individual meiocytes will not result in cell cohesion because of persistence of meiocyte wall.

Usually callose is deposited between cellulosic wall and the membrane of the meiocytes, the former disintegrates during meiosis. Later, only the callose envelope encloses meiocytes. But the presence of acellular deposition in the tapetal cells, which later, in addition to callose, locates between tapetum and meiocytes in *D. ovatum* is an unusual feature and is considered as an abnormal feature. In EMS-treated triticale anthers the deposition of callose is reported in tapetal cells (Bhandari and Khosla, 1995). It is interpreted by these authors that the secretion of callose is not confined only to meiocytes and the precursors for callose synthesis are formed in the tapetum and are released in the locule for utilization by the meiocytes at the appropriate timings. Since, in *D. ovatum* (present study), the occurrence of autofluorescent deposition in the tapetum and its release into the locule is prior to
callose synthesis, it is hypothesized that the tapetally derived deposition contains callose precursors, in addition to adhesive elements required to bring aggregation of pollen tetrads. However, the anthers of *D. ovatum* deviate from the anthers of majority of angiosperms, including *M. oliefera*, in showing callose deposition only on the outer surface of peripherally located meiocytes (present study). Such pattern of callose deposition also effectively isolates the entire mass of meiocytes. The lack of callose deposition around the other meiocytes may be considered as an adapted character to facilitate the formation of pollinia, because, presumably, the absence of callose helps in the persistence of meiocyte primary wall which holds meiocytes in group as well as prevents them from forming plasmodial mass, as it happens in rice (Cebrat and Zadecka, 1978) and EMS-treated triticale (Bhandari and Khosla, 1995).

Thus, the above observations amply suggest that, at the time of initiation of meiosis, meiocytes require some sort of protective cover. This is achieved through different mechanisms. In the majority of angiosperm anthers, including *M. oliefera*, the isolation
to meiocytes is provided by the deposition of callose around individual meiocytes. In the anthers of *D. ovatum* callose and tapetally derived acellular layer provide required isolation to meiocytes. The persistence of primary wall around individual meiocytes also acts as substitute protective layer. In *D. volubilis* anthers tapetally secreted PAS-positive layer functions as a protective layer and brings isolation to entire mass of meiocytes. These modifications in *D. ovatum* and *D. volubilis* serve as advantageous characters in holding the meiocytes and their derivatives in aggregation, and thus in the formation of pollinium. From economic point of view also, these two plants have achieved the goal of isolation of meiocytes at minimum expense of metabolic energy by laying down the common envelope to entire mass of meiocytes rather than having a protective cover around individual meiocytes.

During pre-meiotic phase, anther wall layers differentiate concurrent with sporogenous tissue and meiocytes. The outer derivative of the archespor cell- the primary parietal cell- divides periclinally to produce outer parietal cell and inner tapetal cell
A concentric ring of tapetal tissues is formed around the sporogenous cells, as bordering cells of the connective also differentiate into tapetal cells. Tapetum differentiates at sporogenous stage, becomes metabolically very active during meiosis, and degenerates at late microspore stage.

Structurally and histochemically tapetal cells have close resemblance to sporogenous cells. Cell ablation experiments have shown that formation of tapetal tissue is an indispensable requirement for normal pollen development (Koltunow et al., 1990). Generally, the tapetal cells are non-vacuolate, except in *M. oliefera*, and densely cytoplasmic, rich in RNA and proteins (Panchaksharappa et al., 1985; Chapman, 1987; Shah et al., 1991; present study). In *M. oliefera*, the single large vacuole breaks into several small vacuoles (present study). This perhaps increases the synthesis of membrane system and enhances the absorption potential of the tapetum. Cell walls of tapetum are extremely thin with a negligible deposition of cellulose (Bedinger, 1992; Loukides et al., 1995; present study). In tomato, the wall between adjacent...
tapetal cells and between tapetum and sporogenous cells is simple and fibrillar (Polowick and Sawhney, 1993). During the period of callose deposition and meiosis, the wall fibrils loosen and appear fibrous with granular inclusions. According to Polowick and Sawhney (1993) these changes in wall structure facilitate the transport of material into the locule. In this context, the tapetal wall structure in *D. volubilis* and *D. ovatum* is exceptional because in former tapetal cell walls are thick and rich in general polysaccharides, whereas in the latter the wall between the tapetum and sporogenous cells rich in cellulosic deposition (present study). The presence of ascorbic acid along the tapetal cell wall suggests its role in the biosynthesis of wall material. This possibly suggests the transitory loss of secretory nature of the tapetal cells in these plants. In the absence of callose deposition around the individual meiocytes, this unusual feature of the tapetal cells in *D. volubilis* and *D. ovatum* helps in creating isolation of meiocytes. At the same time, the thick-walled tapetal cells may impede the translocation of nutrients into the locule. However, in these plants meiocytes may not depend upon tapetally-supplied metabolites since they are self-sufficient by virtue of presence of storage cytoplasmic.
polysaccharides. The reduction in the cytoplasmic polysaccharides in dyads and tetrads favors this presumption.

During meiosis, tapetum attains peak metabolic potential as evidenced by its rich contents of RNA and proteins in all the three plants studied and also in ascorbic acid in *D. volubilis* and *D. ovatum* (present study). Similar reports are available on the tapetal cells of other plants (Bhandari *et al.*, 1976; Chewrot and Gorska-Brylass, 1981; Sheel and Bhandari, 1990). The tapetal cells of *M. oliefera* show some exceptional features, which are uncommon in other angiosperms. At meiocyte stage the tapetal cells in *M. oliefera*, especially the inner tapetum, enlarge and contain one large vacuole at their outer face. The cytoplasm accumulates towards the meiocytes. Though speculative, the significance of establishment of polarity might be related to more effective nutritional correlation between tapetum and meiocytes.

Another unusual feature of tapetum in *M. oliefera* is the presence of starch storage in it at meiocyte stage. According to Pacini *et al.* (1992), upto meiosis, both tapetal and reproductive

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cells contain proplastids. After meiosis, proplastids in microspores differentiate into amyloplasts, whereas in the tapetal cells they differentiate into specialized type of chomoplasts called elaioplasts (Polowick and Sawhney, 1990; Pacini et al., 1992). The occurrence of starch grains in the tapetal cells of *M. oliefera* anther suggests the premature conversion of proplastids into amyloplasts. However, the degradation of starch storage in the tapetal cells, during meiosis, suggests the availability of carbohydrate resources needed to enhance the metabolic potential of the tapetum. In this respect, tapetum of *M. oliefera* is different from the tapeta of *D. volubilis* and *D. ovatum*. The transitory storage of carbohydrate in the tapetum of *M. oliefera* is an example of the tissue's ability to elaborate the metabolites from the parent plant and to function as sink organ up to the appropriate moment of release or reabsorption of nutrients.

The metabolic hyperactivity of the tapetum during meiosis is evident by the rich presence of mitochondria, plastids, dictyosomes, endoplasmic reticulum and ribosomes (Moss and Heslop-Harrison, 1967; Lee and Warmke, 1979; Hallden et al., 1991; Bedinger, 1992; Polowick and Sawhney, 1993). In addition, tapetal cells also
possess peak activities of non-specific esterases and acid phosphatase (Vithanage and Knox, 1979) and adenosine triphosphatase, glucose-6-phosphatase, cytochrome oxidase, succinic dehydrogenase and malate dehydrogenase (Hegde and Andrade, 1982; Andrade and Hegde, 1983; Hegde and Isaacs, 1992). Therefore, the tapetum functions as a transient nutritive tissue (Chapman, 1987; Regan and Moffatt, 1990; Shah et al., 1991). In addition to providing nutrition to reproductive cells (Pacini and Franchi, 1983), tapetum is also involved in the regulation of microspore development (Mariani et al., 1990; van der Meer et al., 1992) and in the synthesis and secretion of various lipophilic substances the pollenkitt and trypnine (Weber, 1992). In tritiacale (Bhandari and Khosla, 1995) and D. ovatum (present study) presence of callose precursors in the tapetal cells indicates that they are also source of callose.

The anther wall layers and connective also participate in the production of healthy viable reproductive cells. During pre-meiotic phase, occurrence of starch storage in the connective and wall layers is reported in M. oliefera (present study) and several other
angiosperm anthers (Reznickova, 1978; Cheng et al., 1979; Reznickova and Willemse, 1980; Bhandari and Khosla, 1982; Bhandari and Sharma, 1983; Andrade and Hegde, 1983; Vijayaraghavan, Kumara and Sujata, 1987; Sheel and Bhandari, 1990; Hegde and Isaacs, 1992; Hegde et al., 2000). The absence of starch storage in the anthers of D. volubilis and D. ovatum (present study) is considered as an indication of its utilization in the production of energy and metabolic substances for the differentiating reproductive cells (Vijayaraghavan and Cheema, 1978; Vijayaraghavan and Sudesh, 1994). Synthesis and degradation of carbohydrates, at specific developmental stages of anther, indicate that the formation of mature fertile male gametophyte depends on nutrients provided by the anther sporophytic tissues. Deficiency in carbohydrate metabolism in the anther leads to abnormal pollen development (Sawhney and Bhadula, 1988; Bhadula and Sawhney, 1989; Sawhney, 1992) and is often correlated with male sterility (Banga et al., 1984).

Physiological studies have shown that, because of their growth intensity and metabolic activity, anthers possess highest
sink strength among floral parts and therefore attract largest proportion of nutrient uptake (Lawrence and Mayne, 1991; Clement et al., 1994). In *Lilium*, between meiocyte stage to vacuolate microspore stage, the intense growth of the anther coincides with starch breakdown and anther consumes entirely its amylaceous reserves (Clement et al., 1996). Presumably, this is the reason why starch storage is not observed in the anthers of *D. volubilis* and *D. ovatum* (present study). The presence of starch storage in the connective and wall layers of the *M. oliefera* anthers suggests that part of the sucrose supplied by the filament is utilized for starch synthesis. Thus, the connective and wall layers serve as sink organs and represent potential source of carbohydrates for the biosynthetic activities in anther tissues. Utilization of carbohydrates has been implicated in the synthesis of ammonia precursors of lipids, carotenoids, sporopollenin (Atkinson et al., 1972; Reznickova and Willemse, 1980) and metabolites such as ascorbic acid (Chinoy et al., 1971; Andrade and Hegde, 1983; Vijayaraghavan, Kumara and Sujata, 1987).
MEIOTIC PHASE:

Whether callosic deposition occurs or not, meiocytes undergo normal meiosis and cytokinesis in the plants studied in the present investigation. In *D. volubilis* and *D. ovatum* the primary wall of meiocytes persists during meiosis, but not in *M. oliefera* (present study). Persistence of primary wall of meiocytes is also observed in *Allium tuberosum, Cyclamen persicum* (Bhandari et al., 1981) and *Allium sativum* (Gori, 1983). According to Chang and Neuffer (1989) the meiocyte wall has great significance in the meiotic event since it forms the compact framework that remains unattached to the inner cell protoplasm during meiosis. As a result, a large space is provided for the completion of meiosis without outside disturbance. However, in *M. oliefera* primary wall of meiocytes is not persistent and therefore it appears that its presence is not indispensable for the completion of meiosis (Horner and Rogers, 1974; present study). In *D. ovatum* the persistent primary wall of meiocytes holds microspores in tetrads (present study).

Meiocytes in *D. volubilis* show reduction in cytoplasmic RNA and protein contents prior to meiosis. This feature appears to be a
general phenomenon in several angiosperm anthers (Pacini et al., 1985). Moss and Heslop-Harrison (1967) and Vijayaraghavan and Cheema (1978) consider the dilution of cytoplasm, due to enlargement of meiocytes, as a cause for the reduction in RNA and proteins. Knox et al. (1971) attribute breakdown and elimination of ribosomes in meiocytes to loss of RNA, whereas blockade in RNA synthesis (Porter et al., 1984) and presence of specific nuclease in meiocytes (Dickinson, 1992) are also considered responsible for reduction in RNA.

Reduction in cytoplasmic RNA and proteins during meiosis is considered as a significant event. By eliminating cytoplasmic RNA meiocytes reorganize their cytoplasm so that sporophytic influences are eliminated and suitable environment is created for gametophytic expression (Bird et al., 1983; Rodkiewicz et al., 1986; Dickinson, 1987).

In M. oliefera and D. ovatum meiocytes do not show reduction in cytoplasmic RNA and proteins (present study). Yet normal meiosis and cytokinesis occur in the anthers of these plants.
It means that meiocytes in these plants reorganize at ultrastructural level, which is not recognizable by LM studies. For instance, the reorganization of meiocytes in *Catananche* occurs in the form of changes in the ER structure (Blackmore and Barnes, 1988). Reorganization is also observed in the differentiation of plastids and mitochondria (Heslop-Harrison, 1971; Dickinson, 1982). Meiocytes in *Solanum nigrum* (Bhandari and Sharma, 1988) and *Carica papaya* (Sheel and Bhandari, 1990) show movement of granular inclusions during meiosis. In *Lilium* meiosis is associated with disintegration of cytoplasmic nucleoloids, which are formed by aggregation of DNA transcripts (Sato *et al*., 1991). After disintegration of nucleoloids, meiocytes are repopulated with ribosomes. Thus, reduction in cytoplasmic RNA and proteins is not a prerequisite condition for the initiation of meiosis.

EM studies reveal that, during meiosis, meiocytes are interconnected with cytomictic channels through the pores present in the callose and therefore meiotic prophase occurs in coenocytic conditions (Dickinson, 1992). But according to Hallden *et al.* (1991), in *Beta vulgaris*, plasmodesmata disappear between
meiocytes at prophase-I. In this plant, at prophase, meiocytes show increase in number and decrease in size of mitochondria and plastids, increase in ribosomes and ER and occurrence of small vacuoles. In sunflower, association of mitochondria with nucleus, during the later stages of meiosis, is considered to be a mechanism that ensures equal distribution of mitochondria between the four microspores produced following the meiotic division (Smart et al., 1994). Riggs and Hasenkampf (1991) and McCormic (1993) report the occurrence of few meiosis-specific proteins that play a role in chromatin packing.

During meiosis, the tapetal cells are rich in RNA and proteins. Starch storage present in the tapetal cells of *M. oliefera* and cytoplasmic polysaccharides in the tapetal cells of *D. volubilis* and *D. ovatum* deplete during meiosis. Reduction in the polysaccharides is also noticed in the meiocytes during meiosis. These observations imply that the carbohydrates present in the tapetal cells are utilized to boost their own metabolic potential. Meiocytes presumably depend on their own storage metabolites.
POST-MEIOTIC PHASE:

During this phase differentiated anther switches from a histodifferentiation program to a cell degeneration and dehiscence program that leads ultimately to pollen release and stamen senescence at flower opening. The dehiscence program begins after the formation of tetrads, resulting in the sequential destruction of specific anther cell types, and is coordinated temporally with the pollen differentiation process.

Completion of meiosis and microspore tetrad formation is marked by the regain in the synthesis of RNA and total proteins in them (Mandaron et al., 1990; present study). According to Dickinson and Heslop-Harrison (1977) conversion of chromosome-associated ribosomal RNA into residual RNA and restoration of ribosomal population are responsible for increase in cytoplasmic RNA and proteins in tetrads. In addition, except in *D. volubilis*, microspores in tetrads also possess rich cytoplasmic polysaccharides (present study). Presence of these metabolites in young microspores is suggestive of their ability to grow and develop.
In anthers that produce pollen grains in monads, including *M. oliefera*, microspore tetrad phase terminates when the callosic deposition is lysed enzymatically by tapetally-secreted callase. Microspores become free after the dissolution of callose (Fitzgerald *et al.*, 1993b; present study). In many male sterile plants persistent callose deposition prevents the separation of microspores from tetrad condition (Horner and Rogers, 1974; Horner, 1977; Regan and Moffatt, 1990; Theis and Robbelen, 1990; Hegde and Isaacs, 1992; Kini *et al.*, 1994; Abad *et al.*, 1995; Wei *et al.*, 1996). In *D. volubilis* anthers (present study), soon after cytokinesis, microspores separate because of absence of callose and disintegration of meiocytes wall. In *D. ovatum* pollen grains remain in tetrad condition even in the mature anther because of persistence of meiocytes wall. Similar condition occurs in *Spathoglottis plicata* (Prakash and Lee-Lee, 1973; Hegde *et al.*, 2000). In transgenic tobacco also microspores are held in tetrads in the absence of surrounding callosic wall because of existence of cellullosic tetrad wall (Worrall *et al.*, 1992).
In polyad systems compound pollen grains are formed because of cohesion between microspores, which results from different mechanisms. Knox and McConchie (1986) describe two types of mechanisms: (a) presence of a common tectum surrounding the entire polyad or each pollen grain with its own tectum but fused with adjacent grain (simple cohesion) and (b) presence of wall bridges connecting the individual spores (cross wall cohesion). According to Yeung (1987) pollen grains of *Epidendrum ibaguense* are held together due to combined effect of several features such as: incomplete removal of cell walls of meiocytes, persistence of callosic cell walls around tetrads, persistence of plasmodesmata from meiocyte stage, incomplete cell wall formation within the tetrad and failure to increase in size of pollen grains. In *Acacia paradoxa* Fitzgerald *et al.* (1993b) report contact of endexine of adjacent pollens through bridges located in the germinal apertures. In another species of *Acacia*, 16 microspores present in each locule are held within the callosic wall at tetrad stage (Kenrick and Knox, 1979). Callose wall is perforated by the cytoplasmic connections linking the inner faces of microspores. These cytoplasmic connections determine the sites of
exine wall bridges found in mature polyads. At maturity polyads are held within a membranous sac lined with orbicules. This ensures efficient dispersal of the polyad (Kenrick and Knox, 1979). Tetrads of *Pyrola japonica* are surrounded by a continuous tectum (Takahashi and Sohma, 1980). In *Typha latifolia*, tetrads cohere as a result of fusion of the tectum on the inner faces between neighboring pollen grains (Takahashi and Sohma, 1984). The tectum is initiated at the time of primexine deposition and ektexine differentiation, prior to the dissolution of the callose (Blackmore *et al.*, 1987). In *Oenothera interovan* fibers connect the developing ektexine with the surface of the tapetal cells immediately after the dissolution of callose wall (Takahashi and Skavarla, 1990). Pollen aggregation through sticky, viscous pollenkitt or through non-sticky, non-viscous exinal connections and viscin threads also has been implicated (Chaudhry and Vijayaraghavan, 1995). In *Simmondsia chinensis* pollen aggregation is effected by acetolysis-resistant thread-like exinal connections (Chaudhry and Vijayaraghavan, 1995). In *Pholidota* a thin, transparent PAS-negative substance, presumably callose is present all around the pollen aggregate (Arora and Kapil, 1989). The adjacent pollen
grains in the pollinium are joined together by means of short, thin exinous linkages.

In *D. volubilis* the microspores are held together due to combined effect of at least two features. The individual microspores are surrounded by wall material, which is polysaccharide, cellulosic and lipoidal in nature. Lipoidal nature of the wall is also reported in *Asclepias curassavica* (Woodson, 1954). Initially, this wall is thin. The adjacent microspores in the pollinium are cemented together by the fusion of microspore walls. As the pollen grains mature this wall becomes thick. The wall synthesis around individual microspores is presumably associated with the presence of ascorbic acid because ascorbic acid is seen along the spore wall. The acetolysis nature of wall also implicates its sporopollenin nature.

In addition to cohesion of individual microspores, the formation of tapetally secreted acetolysis-resistant layer also assists the formation of pollinium in *D. volubilis* (present study). This PAS-positive layer initiates, at meiosis, on the inner face of the tapetum. Woodson (1954) reports that in *Asclepias curassavica*
such layer is secreted exclusively by the outer tapetum. During the process of differentiation of pollen grains, this layer extrudes from the tapetum and deposits on the surface of the pollen aggregate. This pollinial coat is of uniform thickness and acetolysis resistant. It is autofluorescent and azure B/toluidine blue and PAS-positive. This probably suggests that the pollinial coat is composed of sporopollenin, lignin, cutin or bound form of lipids (Cave and Bell 1974). But lipids are not autofluorescent whereas sporopollenin produces a greenish-yellow autofluorescence (van Gijzel, 1971). Sporopollenin also responds positively for lignin with azure B (Southworth, 1973, 1974; Vijayaraghavan and Shukla, 1976). Therefore, as inferred by Vijayaraghavan and Shukla, (1976) in *Pergularia daemia*, the pollinial coat in *D. volubilis* is predominantly composed of sporopollenin. Formation of acetolysis resistant extra tapetal membrane has been reported in other plants also. Heslop-Harrison (1967) reports such membrane in the plasmodial tapetum of Compositae. In *Sorghum bicolor* all the orbicules fuse to form an orbicular wall on the locule surface (Christensen *et al.*, 1972). In *Simmondsia chinensis* inner face of the tapetum is deposited with sporopollenin (Chaudhry and Vijayaraghavan, 1995). According to
Keijzer (1987), in entomophilous species, a continuous coating of sporopollenin on the inner tangential wall of the tapetum increases the efficiency of pollen dispersal. In tomato, the orbicules remain on the surface of the degenerating tapetum and forms a continuous boundary, which may form a possible barrier to the entry of pathogens into the locule (Polowick and Sawhney, 1993).

Unlike *D. volubilis*, pollen grains in *D. ovatum* are in tetrads. Individual pollen grains possess very thin PAS-positive, cellulosic and lipoidal wall (present study). The persistent primary wall of meiocytes constitute the tectum of pollen tetrads, which is also PAS-positive, cellulosic and lipoidal in nature. The cohesion of adjacent pollen tetrads is not effected by the fusion of wall of tetrads. Instead, it seems that the pollen tetrads are held together by means of the autofluorescent, PAS-positive substance secreted by the tapetal cells. According to Cocucci and Jensen (1969), in orchids, adherence of tetrads to one another results in two micro-environmental conditions. One of them is at the pollinium surface, where the tetrads are in permanent contact with the tapetum. But in *D. ovatum* such contact between the tapetum and pollinium
surface is not observed (present study). The second is inside the pollinium where tetrads are in touch with only other tetrads. This feature also is not observed in the present study. According to Yeung (1987), thin walls of the central pollen tetrads not only increase their chances of germination but help in pollen aggregation. Formation of an elaborate exine may disrupt the existing connections within the tetrads.

In some members, the pollen coat (pollenkitt, tryphine, elastoviscin) formed on the pollen surface is responsible for the formation of pollinium. The pollen coat, synthesized in the tapetum, is primarily lipidic but presence of other compounds is not ruled out (Fitzgerald et al., 1994). In *Pterostylis* pollen coat is opaque, whereas in *Dendrobium* pollen coat is released as electron lucent drops that become an electron-opaque cuticular layer by anthesis (Fitzgerald et al., 1994). In *Pterostylis* the final tapetal secretory event releases lipidic material, which surrounds the entire anther locule. According to Fitzgerald *et al.* (1993b), this is the future pollen glue material and is released before microspore mitosis, which facilitates all the pollen grains to have pollen coat.
In *Dendrobium* the release of pollen coat occurs after the microspore mitosis, which results in the coating of pollen glue only on the outer pollen grains (Fitzgerald *et al.*, 1994). The continual turnover of water in the anther locule (supplied by the vascular strands and removed by the transpiration) would generate fluid movement, mobilizing immiscible lipidic tapetal secretions, allowing drops of pollen coat to be passively distributed throughout the pollinium (Fitzgerald *et al.*, 1994). The glue oozes down between tetrads forming bridges between the tetrads and effectively encasing the entire pollinium. When the flower bud opens the pollen glue becomes hard by polymerization brought out by the exposure to air and light. Operation of similar mechanism is presumed in the formation of pollinium in *D. ovatum* (present study). Because, in this plant, tapetum releases autofluorescent PAS-positive secretion which covers the outer meiocytes. The secretion is also azure B and toluidine blue positive and contains ascorbic acid. After meiosis, this secretion, as detected by autofluorescence, is observed in the locule, between microspore tetrads. At maturity this secretion lacks autofluorescence indicating the change in its chemical nature. The presence of lipid deposition on the tetrad tectum as well as on the
walls of the pollen grains suggest the tapetal secretion is lipoidal and functions as pollen glue material (present study). In *Epidendrum scutella* a fibrous wall layer, present both in outer and inner tetrads, holds the tetrads together. The fibrous layer is identified as nexine (Cocucci and Jensen, 1969).

In the plants, which produce pollen grains in monads, such as *M. oliefera* (present study), the tetrad stage is most significant with regard to the formation of pollen wall (Fernandez and Rodriguez-Garcia, 1988; Chaudhry and Vijayaraghavan, 1996). Pollen wall is both gametophytic and sporophytic in origin. The two major phases of exine ontogeny include template formation (in callose bound microspores) and sporopollenin deposition (in free microspores). In *M. oliefera*, soon after meiosis is completed, synthesis of pollen wall begins (present study). In most plants, including *M. oliefera*, primexine is patterned while the microspores are still invested within the callosic wall (Kronestedt-Robards and Rowley, 1989; McCormic, 1993; Vijayaraghavan and Sudesh, 1994; Chaudhry and Vijayaraghavan, 1995, 1996; present study).
Circumstantial evidences point out that callosic wall has a role in exine development as a glucose source or as a stress factor in the process of compression and flattening of the upper ends of the rod like probacula to form tacti (Vijayaraghavan and Shukla, 1977). Earlier and present results support the contention that the callose wall acts as a framework and provides a template or mold for exine wall formation (Waterkeyn and Beinfait, 1970,1971; Chaudhry and Vijayaraghavan, 1996). In Epacridaceae non-elaborated fragmentary exine is formed at the sites where callose deposition is less (Ford, 1971). In *Pergularia daemia* failure or poor formation of microspore wall correlates with the absence of callose around tetrads (Vijayaraghavan and Shukla, 1977). In transgenic tobacco, premature dissolution of callose leads to the formation of abnormal exine and bursting of microspores (Worrall *et al.*, 1992). In many male sterile anthers premature or delayed dissolution of callose affects the pollen wall formation (Horner and Rogers, 1974; Horner, 1977; Graybosch and Palmer, 1987; Hegde and Isaacs, 1992). In *D. volubilis* also, lack of callose deposition correlates with formation of poorly developed wall without well-defined exine (present study). The importance of presence of callosic deposition at
tetrad stage is still better exemplified by *Spathoglottis plicata* (Hegde et al., 2000) and *D. ovatum* (present study). In the anthers of these plants only the peripheral meiocytes/microspore tetrads possess partial callose deposition only on their outer face. Consequently exine formation occurs only at the site of callose on the peripheral pollen tetrads.

These speculations, based on the circumstantial evidences, are of little help in understanding the relationship between callose deposition and pollen wall formation. As there are reports suggesting the requirement of callose deposition for the proper formation and surface patterning of the exine, the reports are also available indicating exine pattern is not determined solely by callose. In *Poinciana gilliesii* (Skavarla and Rowley, 1987), *Hibiscus syriacus* (Takahashi and Kouchi, 1988), *Caesalpinia japonica* (Takahashi, 1989, 1993) and *Bougainvillea spectabilis* (Takahashi and Skavarla, 1991) exine pattern is determined not by callose but by the mosaic differentiation of the plasmamembrane. In the anthers of space-flight grown *Arabidopsis thaliana*, despite of weak deposition of callose and early degeneration of tapetum, the sterile
empty young microspores possess well-developed normal-looking exine (Kuang et al., 1995).

The free microspores, in many angiosperm anthers, come in contact with tapetally-derived sporopollenin. The sporopollenin undergoes polymerization and forms acetolysis-resistant mature exine wall (Chaudhry and Vijayaraghavan, 1996). The polymerization of sporopollenin from its precursors (carotenes and carotenoid esters) and its subsequent incorporation into exine involve activity of various enzymes (Sawhney and Bhadula, 1988). In sunflower, the enzymes transferred from the tapetum include esterases and acid phosphatase. Accumulation of tapetal esterase commences at spore-release period and reaches maximum around mid-vacuolate microspore period (Vithanage and Knox, 1979). After tapetal dissolution esterase accumulates on sexine spines of pollen surface. The outer exine is sporopollenin in nature. Esterase present in pollen aperture may function as a cutin-hydrolyzing enzyme and may play a vital role in self-incompatibility reactions on the stigma. In Lycopersicon also, esterases are present in the tapetum before its degeneration and on the microspores at the time
of exine deposition (Sawhney and Nave, 1986). In *prosopis juliflora*, sporopollenin deposition is first synthesized by the microspore protoplast at tatrad period within the callosic wall (Chaudhry and Vijayaraghavan, 1996). Later, at young spore period, deposition of sporopollenin is contributed by the tapetal cells. Thus, the principal structural features of the exine pattern are established within the callosic wall at late tetrad stage and edification and uniform electron density of the ektexine is achieved when the microspores are released from the tetrads (Chaudhry and Vijayaraghavan, 1996).

Tapetum constitutes a major source of exine materials. Sporopollenin bodies have been associated chiefly with secretory tapetum. In *Butomus umbellatus* sporopollenin bodies are reported in the plasmodial tapetum (Fernando and Cass, 1994). At 3-celled stage, the part of disintegrated periplasmodium deposits on the exine as tryphine (Galati, 1996).

In *prosopis juliflora* (Vijayaraghavan and Chaudhry, 1993; Chaudhry and Vijayaraghavan, 1996) and *M. oliefera* (present
study) concurrent with primexine formation, tapetal cells show extrusion of pro-orbicular bodies (pro-Ubisch bodies). The pro-Ubisch bodies and exine in the vacuolate microspores stain moderately for PAS and proteins. In *Triticum* exine and pro-Ubisch bodies are rich in acidic polysaccharides, neutral polysaccharides and unsaturated lipids (El-Ghazaly and Jensen, 1987). The stainability for polysaccharides in exine and Ubisch body, as well as lipids in exine increases in mature pollen grains (El-Ghazaly and Jensen, 1987; present study). Ubisch body also shows the presence of sporopollenin. According to Heslop-Harrison and Dickinson (1969) and Shoup *et al.* (1980) tapetal cells contribute sporopollenin precursors to the final assembly and/or modification of exine. Role of endoplasmic reticulum is implicated in the production of sporopollenin (Barnes and Blackmore, 1988).

In *D. volubilis* anthers also a wall, comparable to primexine, is synthesized around each microspore (present study). However, unlike in *M. oliefera*, the PAS-positive, lipidic and cellullosic wall of the microspores is synthesized in the absence of callose and after the microspores separate from the tetrads. In *Pergularia daemia*
also the microspore wall is cellulosic (Vijayaraghavan and Shukla, 1977). This wall is also autofluorescent and acetolysis-resistant, and therefore sporopollenin in nature. In this respect microspore wall resembles the pollinial coat (present study). But microspore wall is not derived from the tapetum, because the synthesis of microspore wall occurs at the time when the precursors of wall material from the tapetum are not yet extruded from the tapetum. This observation supports the view of Chaudhry and Vijayaraghavan (1996) that initial sporopollenin deposition on the microspore wall originates in microspore protoplasm and therefore is gametophytic in origin. Meanwhile, the tapetum secretes PAS-positive wall material on its inner surface, which later acquires autofluorescence (present study). In this way the process of synthesis of sporopollenin-precursors in the tapetum of *D. volubilis* is comparable to the secretion of pro-Ubisch and Ubisch bodies in *M. oliefera*. But in the former the sporopollenin deposition is in the form of continuous layer, which forms a pollinial coat around the entire aggregate of microspores. Thus, in *D. volubilis* elaboration of pollen exine does not occur because tapetally derived sporopollenin precursors fail to deposit on the surface of individual pollen grains.
Poor elaboration of exine appears to be a common feature in Asclepiadaceae members (Vijayaraghavan and Shukla, 1977; Bendigeri, 1999). In *Pergularia daemia* pollinial coat is present around the aggregate of microspores, but not on the walls that surround the individual spore. Therefore, Vijayaraghavan and Shukla (1977) are of the opinion that individual microspores have lost the ability to synthesize the exine material. The exine lacks the characteristic structure and is represented by thin and often fragmented lamellae (Vijayaraghavan and Shukla, 1977).

In *D. ovatum*, only the peripheral pollen grains possess, partial exine wall that is comparable to the exine of monad pollen grains (present study). In *Spathoglottis plicata* also the exine wall is incomplete and confines to the outer face of the pollen tetrads (Hegde et al., 2000). In the anthers of *M. oliefera* and others, which produce monad pollen grains, primexine development begins when the microspores are still in callose-bound tetrad stage. As indicated earlier, the sporopollenin deposition on the primexine occurs when the spores are freed from the tetrads. In *D. ovatum* also individual microspore possesses weakly PAS, lipid and cellulose positive wall.
when they are in tetrad condition. Hegde et al. (2000) envisage that, in *Spathoglottis plicata*, the degraded products of carbohydrates present in the locule are utilized for the synthesis of microspore walls. In *D. ovatum* (present study) and *Spathoglottis plicata* (Hegde *et al.*, 2000) the exine wall on the outer surface of peripherally located microspore tetrads is formed because of presence of callose deposition at that site during meiocytes and young tetrad stages. Later, the callose deposition disintegrates and the wall material deposited on the peripheral pollen tetrads becomes autofluorescent indicating the presence of sporopollenin. In addition to sporopollenin test, the production of green colour to Azure B/toluidine blue tests by the outer wall further strengthens the view that the wall is sporopollenin in nature.

In *Epidendrum scutella* sporoderm of outer tetrads has a sexine and an intine while that of inner tetrads lacks sexine, and intine is formed only after the pollinium is on the stigma (Cocucci and Jensen, 1969). It is implicated that the tapetally derived sporopollenin material reaches only the superficially located tetrads. In *D. ovatum* (present study), *Epidendrum scutella* (Cocucci
and Jensen, 1969) and *Spathoglottis plicata* (Hegde *et al.*, 2000) it is assumed that the tapetum secretes only limited quantity of sporopollenin precursors. In male sterile mutants of tomato similar condition is reported by Sawhney and Bhadula (1988). In this mutant, very low activity of esterases is recorded and only those microspores lying close to the tapetum receive the sporopollenin precursors and the enzyme responsible for exine deposition (Sawhney and Bhadula, 1988). In *D. ovatum* also the possibility of low activity of esterases is responsible for limited exine development on outer microspore tetrads needs verification.

A correlation between the absence or poor development of exine and pollen abortion has been envisaged in many male sterile plants (Horner and Rogers, 1974; Horner, 1977; Graybosch and Palmer, 1987; Theis and Robbelen, 1990; Hegde and Isaacs, 1992; Katti *et al.*, 1994). It is paradoxical that in Asclepiadaceae and Orchidaceae, including *D. volubilis* and *D. ovatum*, despite the absence or poor development of exine, fertile pollen grains are produced.
Several male sterile lines of maize are defective in pollen wall biosynthesis, a finding consistent with the proposed role of the tapetum in exine production (Bedinger, 1992). But absence or poor development of pollen exine in *D. volubilis* and *D. ovatum* is apparently not due to malfunctioning of the tapetum (present study). As in normal anthers of angiosperms, in *D. volubilis* and *D. ovatum* tapetum secretes sporopollenin layer on its inner tangential surface. In *D. volubilis* this sporopollenin layer extrudes from the tapetal surface and forms a continuous pollinial coat which surrounds the aggregate of microspores (Vijayaraghavan and Shukla, 1977; present study). Thus, only deviation observed here is the failure of sporopollenin precursors to deposit on the surface of individual microspores. If one considers the aggregate of microspores as a single unit, and therefore equivalent to single pollen grain, then the pollinial coat can be considered equivalent to pollen exine. The ontogeny and chemistry of pollinial coat substantiates this contention. In monad pollen grains activities of certain enzymes, such as esterases and acid phosphatase are located in the pollen wall (Vithanage and Knox, 1979). In *Asclepias curassavica* activity of these enzymes is localized in the pollinial
coat (Bendigeri, 1999). The pollen wall proteins play an important role in self and inter-specific incompatibility system. Upon pollination, it is the pollinial coat that comes in direct contact with the stigmatic tissue. In *D. ovatum* (present study) and *Spathoglottis plicata* (Hegde et al., 2000) the peripheral pollen tetrads, which possess exine wall on their outer face, come in contact with the stigmatic tissue. There are also some structural similarities between the pollinial coat and pollen wall. In *Calotropis gigantea*, although it was thought that the pollinial coat is uniformly thick all along the entire length (Viswanathan and Lakshmanan, 1986), the ultrastructural studies have revealed the presence of apertures of various sizes and shapes on the pollinial coat (Sreedevi and Namboodiri, 1982). In *Asclepias* presence of tiny notches on the pollinial coat marks the germination site (Galil and Zeroni, 1969). Through this region water enters and hydrates the pollen grains facilitating their germination. Germinal furrow region is inserted into the stigmatic chamber on pollination. Germination of the pollinium is asymmetrical because pollen tubes emerge from the convex side of the pollinium, even when it is thrust into the stigmatic chamber in a reversed position. According to Galil and
Zeroni (1969) polarity of the pollinium is some way connected with the arrangement of sporogenous cells. Thus, not only being tapetal in origin, the pollinial coat temporally, structurally, chemically and functionally exhibits similarity with exine of individual pollen grains. Therefore, in polyads, the individual pollen grains can dispense with exine walls because separately they never come in direct contact with outer environment. Thus, it is the function of pollinial coat (D. volubilis) or peripherally located pollen tetrads (D. ovatum) to associate with pollen-pistil interaction.

During post-meiotic phase anther switches from a histo-differentiation program to a cell degeneration and dehiscence program that leads ultimately to pollen release and senescence at flower opening. After the formation of tetrads sequential destruction of specific anther cell types occur which is coordinated temporally with the pollen differentiation process. One of the anther tissues that degenerate is tapetum.

In addition to sporopollenin precursors, the degenerating tapetum also provides various other metabolites for the pollen
development (Mascarenhas, 1975; Bhandari, 1984; Chapman, 1987). In *Ledebouria socialis* cytoplasmic degeneration of the tapetal cells occur after pollen mitosis (Hess and Hesse, 1994). After callose dissolution tapetal cells show intense exocytosis of polysaccharides into the anther locule.

In *Carica papaya*, PAS-positive materials present in the tapetal cytoplasm moves into the anther locule and fill it completely, embedding the pollen grains into it (Sheel and Bhandari, 1990). This PAS-positive substance is lost by the anthers when pollen grains become starch rich. According to Sheel and Bhandari (1990) the locular PAS-positive material is used up in the synthesis of storage starch grains present in the pollen grains. In *Asclepias curassavica* the outer tapetum contains numerous lipid bodies, which are secreted into the locule (Woodson, 1954). Subsequently the outer wall of pollinium sac becomes lipoid. So it is the function of outer tapetum to secrete the required lipoid substance, which the walls of the tetrads absorb, while the function of the inner tapetum is purely nutritive (Woodson, 1954). In *M. oliefera*, at young microspore stage, starch grains reappear in
tapetal cells, but are lost during the secretion of Ubisch bodies and accumulation of ascorbic acid. In the tapetal cells of *D. volubilis* and *D. ovatum* also carbohydrate and ascorbic acid are lost subsequent to their degeneration. Therefore, the highly concentrated metabolites of the locular fluid are not merely the remnants from the dissolution of callose and/or the tapetal walls (Hess and Hesse, 1994). Subsequently, the tapetal cells possess abundant endoplasmic reticulum, which are seen in association with osmophilic bodies. The latter contain pollenkitt precursor substances. Pollenkitt precursors are probably flavonoids in nature.

In all the plants studied in the present investigation, tapetum possesses rich contents of RNA and total proteins till its degeneration. The secretory nature of the tapetum is evident from the loss of its primary walls. In *Sagittaria* changes taking place after the dissolution of the tapetal walls during plasmodial formation are considered as indications of reorganization rather than a degenerative process (Galati, 1996). Increase in mitochondria, lipidic globules, differentiation of elaioplasts and vesicles in the tapetal cells are indicative of their high activity (Galati, 1996).
In other plants also rich synthesis of RNA and total proteins, in addition to quantitative increase of mitochondria, are recorded in the tapetal cells (Moss and Heslop-Harrison, 1967; Lee and Warmke, 1979; Chapman, 1987; Chaudhry and Vijayaraghavan, 1995). In *Vigna unguiculata* (Gurrea and Carvalheira, 1994) and *Phaseolus* (Carvalheira and Guerra, 1994) tapetal cells contain polytene chromosomes suggesting increase in DNA amount through endoreduplication cycles. Tapetum is responsible for producing β 1-3 glucanase (callase) that dissolves callose wall and releases haploid microspores from tetrads (Hird *et al.*, 1993). Presumably tapetum secretes callase in *M. oleifera* and *D. ovatum* because in these plants callose disintegrates after meiosis (present study). All these suggest that before they degenerate, tapetal cells acquire one more period of hyperactivity (Kronestedt-Robards and Rowley, 1989).

In the present study it is observed that the loss of cytoplasmic contents of the degenerating tapetal cells correlates with the accumulation of reserve metabolites in the pollen grains. Such correlation is envisaged in other plants also (Pacini *et al.*, 1992).
All these circumstantial evidences indicate the nutritive role of the tapetum. In many male sterile plants male sterility is related to the malnutrition of the pollen grains caused by the malfunctioning of the tapetum (Laser and Lersten, 1972; Gottschalk and Kaul, 1974; Frankel and Galun, 1977; Bhandari, 1984; Hegde and Isaacs, 1992; Katti et al., 1994).

Microspores possess carbohydrates, ascorbic acid, RNA and total proteins (present study). Accumulation of carbohydrates in microspores has been reported in *Pergularia daemia* (Vijayaraghavan and Shukla, 1977), *Carica papaya* (Sheel and Bhandari, 1990), *Psophocarpus* (Vijayaraghavan and Sudesh, 1994), *Spathoglottis plicata* (Hegde et al., 2000) and many other plants (Hegde et al., 1993). During post-meiotic stages, in *M. oliefera*, carbohydrate and ascorbic acid present in the connective and wall layers deplete (present study). This correlates with accumulation of nutrients in the pollen grains and formation of fibrous band thickenings on the endothecial cell. Similar correlation is observed in other plants also (Bhandari and Khosla, 1982; Sheel and Bhandari, 1990). Carbohydrates present in the
mature pollen grains serve as reserve metabolites during germination. According to Mandaron et al. (1990), in *Zea mays*, protein synthesis is low during starch accumulation, but just before anthesis, several basic polypeptides are synthesized which may be required for pollen germination. Utilization of storage carbohydrates in the epidermal cuticle formation has also been assumed (Cheng *et al.*, 1979; Bhandari and Khosla, 1982).

Generally, the middle layer degenerates prior to the degeneration of the tapetum. Except in *M. oliefera* the same situation is observed in the present study. In *M. oliefera* multiple layers of endothecium are formed. After tapetal degeneration formation of fibrous band thickenings occur on the endothecial cell walls. Fibrous thickenings are composed of lignin and suberin (Eames, 1961; Freudenstein, 1991) or cellulose (De Fossard, 1969). In *Acacia* presence of callose is reported in the radial walls of endothecium (Kenrick and Knox, 1979). In *M. oliefera, D. volubilis* and *D. ovatum* endothecial thickenings are lignin and cellulosic in nature.

As mentioned earlier, the differentiation of fibrous thickenings occur after the degeneration of the tapetal cells (Hegde et al., 1993; Katti et al., 1994; Hegde et al., 2000; present study). It is presumed that tapetum releases an inhibitor that prevents endothecial development (De Fossard, 1969; Chauhan, 1977). The degeneration of the tapetal cells results in the non-production of inhibitor substance and consequent development of endothecium.

The onset of the dehiscence programs sets off an ordered series of events within the anther which include (1) the formation of fibrous band thickenings on the endothecial cell walls, (2) degeneration of inter-sporangial septum and merging of the
pollen sacs, (3) breakdown of the tapetum and connective and (4) rupture of the anther at the stomium and pollen release. Male sterile tobacco anthers that lack pollen grains and tapetal cells undergo a normal dehiscence process indicating that dehiscence is not set into motion by signals derived from either the tapetum or differentiating pollen grains (Goldberg, 1993). The dehiscence program requires the activation of many genes, including those that encode hydrolytic enzymes required for cell death, such as RNases, proteases and cellulases.

The function of endothecium is largely undetermined. Although most often suggested role is in anther dehiscence, in *Lycopersicon* (Keijzer, 1987) anthers dehisce despite of the lack of thickened endothecial layer. The reduced thickness of endothecium may reflect its lessened role in effecting pollen dispersal (Freudenstein, 1991).

During the final phase of pollen development, water content of the whole anther decreases. Before dehydration is completed, products of degenerating tapetum tryphine (Pacini, 1992) and
pollenkitt (Keijzer, 1987) are deposited on the pollen grains. In *D. volubilis* they are understandably deposited on the pollinial coat and in *D. ovatum* on the peripheral pollen tetrads. Tryphine and pollenkitt help in clumping of the monad pollen grains and their adhesion to pollinators. In *D. volubilis* anther dehisces by means of vertical germinal furrow, while in *D. ovatum*, anther dehiscence consists of opening of thecae along a linear structure. The stipe or caudicles facilitate the removal of pollinium by attaching to pollinator body.