Chapter - II

Review of Literature
2. REVIEW OF LITERATURE

The aspects of embryology which has been most neglected covers microsporogenesis in hydrophytes and marsh plants. The development of anther in hydrophytes and marsh plants is seldom described in detail, owing to the general impression that the same sequence of events occurs in all angiosperms. It is this trend that has lead to the scanty literature on microsporogenesis, in hydrophytes and marsh plants.

This does not mean that other aspects of reproduction in hydrophytes are not studied. Singh and Sattler (1992) have given the detailed account of floral construction in Alisma and Posluszny and Sattler (1973) in Potemogeton. Eichhornia crassipes is a tristylos system (Mulcahy, 1975). In Eichhornia azurea a correlation exists between tristylos conditions and trimorphic incompatibility (Bianchi et al., 2000). According to Barrett and Harder (1992) Eichhornia paniculata exhibits developmental instability with regard to the position of stamens and elongation of one of the stamens to a position adjacent to the stigma. This results in automatic self-pollination. It is further reported that stamen modification increases in clones grown under
water stress (Barrett and Harder, 1992). Studies by Barrett (1980) have revealed that in *Eichhornia carassipes* sexual reproduction is still in operation despite the propagation is most widespread mode of reproduction. All flowering clones were mid-styled, possessed dimorphic pollen of high viability and pollination success was markedly affected by temperatures below 20°C (Barrett, 1980). Studies on modes of pollination, morphological adaptations for selfing and out-crossing, mechanism of the opening of thecae in water medium and self – compatibility in 12 species of *Potamogeton* have shown that *Potamogeton* sps. have different morphological and functional adaptations for pollination and mating system (Teryokhin et al., 2002). According to Manicacci and Barrett (1995) selfing variants in *Eichhornia paniculata* possess an elongated, short - level stamen adjoined to mid-level stigmas. Pollen from the elongated stamen are larger than pollen from short-level stamens. But alterations in stamen level are not associated with major changes in pollen characteristics (Manicacci and Barrett, 1995). In *Pontederia cordata* long-styled clone experiences legitamate pollination, whereas mid-styled and short-styled clones display random pollination (Glover and Barrett, 1986). In *Eichhornia paniculata* stamen level
depends upon differences in filament length and position of insertion on the floral tube (Richards and Barrett, 1984). In Pontederia cordata a strong pollen trimorphism is associated with differences in stamen and style length (Price and Barrett, 1982). These differences are associated with varying patterns of stamen insertion and development.

2.1 Anther structure in hydrophytes and marsh plants.

Review by Davis (1966) mentions that anthers of hydrophilous plants are tetrasporangiate and wall development follows basic/dicotyledonous/monocotyledonous/reduced type. In some groups epidermis is persistent. Middle layer is ephemeral. Tapetum generally amoeboid. Cytokinesis is successive and rarely simultaneous type. Pollen grains 2 or 3 celled at the time of shedding.

The detailed description of anther structure in Pontederiaceae dates back to 1898 by Smith. In Eichhornia crassipes he reports five layers of wall cells outside the tapetum, the innermost of which disintegrates before the collapse of tapetum. At later stages tapetum becomes binucleate. In mature anther there are two layers in the wall, the inner of which is endothecium with fibrous thickenings.
The microspore mother cells break apart and become rounded. Cytokinesis is successive.

According to studies by Wiegand (1899) on *Potamogeton* hypodermal archesporium, by periclinal divisions, produces three layers. The innermost wall layer is tapetum. Endothecium develops fibrous thickenings. At the onset of meiotic divisions in pollen mother cells tapetal cells undergo complete disintegration. Cytokinesis is successive type. Pollen grains are 2-celled at the time of shedding.

According to Banerji and Gangulee (1937) the mature pollen grains are bi-nucleate in *Eichhornia crassipes* and spermatogenesis takes place in the pollen tube. The generative nucleus is at prophase when it enters pollen tube. Cylokinesis takes place by constriction and two male cells are formed. The vegetative nucleus does not show any sign of degeneration while inside the stylar canal and in pollen tube it precedes the generative nucleus and later the male cells.

The embryological investigations on the two species of *Najas* (Swami and Lakshmanan, 1962) have revealed the dual origin of anther tapetum, which in part originates from the derivatives of the
primary archesporium, and in part from the ground tissue (connective). The tapetal cells are uninucleate and become periplasmodium. Endothecium does not undergo differentiation and there is no definite location of dehiscence. As a result of degeneration of all the wall layers the mature pollen grains lie in immediate contact within the inner envelop of the stamen. Prior to dehiscence, the inner envelop splits open and the pollen grains are scattered in the water medium. Microspore formation is by successive method. The pollen grains are shed at the three-celled stage.

Khanna (1967) reports staminodes in *Victoria.* The anther wall consists of an epidermis, endothecium, 2–3 middle layers in *Victoria* and 3–4 middle layers in *Nymphaea.* In both genera tapetum is secretary type. In *Victoria* tapetum eventually becomes 2-nucleate but later they fuse forming large nucleus. The epidermis gets cutinized in mature anthers and endothecium develops fibrous thickenings. Microspores of a tetrad separate soon after their formation in *Nymphaea,* but they are retained within the mother cell wall in *Victoria.* Some of the compound pollen grains exhibit sterility. In *Nymphaea* pollen grains are heteromorphic—the small grains are non-viable. Microspore nucleus divides to form a large vegetative
cell and a small generative cell. Pollen grains are shed at three-celled stage.

According to Pettitt and Jermy (1975) hydrophilous angiosperms have peculiar pollen grains with reduced structureless exine or without exinous elements. Pettitt, (1976, 1980) has shown that, in three marine plants studied, the pollen grains in *Enhalus* and *Thalassia* have an ornamented exine, but pollen grains in *Halophila* have no exine layer. Thanikaimoni (1978) has proposed a term 'omniaperturate' for the pollen in which the entire pollen wall is made up of a thin exine and thick intine. Martinsson (1993) has shown that exines in some *Callitriche* species tend to be reduced in relation to the submergence of pollination. But SEM and TEM studies on a submerged plant, *Ottelia alismoides*, have revealed verrucate protrusions initiating on microspore plasma membranes at early tetrad stage (Takahashi, 1994). These verrucate protrusions develop into spines during free microspore stage. A foot layer is formed by accumulation of lamellated structure. The pollen grains are inaperturate, not omniaperturate, because of the well developed foot layer. The exine structures in *Ottelia* suggest a low possibility of wholly submerged water pollination (Takahashi, 1994).
In *Sagittaria*, formation of tapetal periplasmodium starts with dissolution of tapetal cell walls during meiotic division (Galati, 1996). The changes which occur after the dissolution of the tapetal wall seem to reflect the reorganization of the tissue. At uninucleate microspore stage the tapetal plasmodium becomes integrated. At three-celled pollen stage the tapetal periplasmodium is disorganized and its remains are observed on the surface of pollen grains. According to Galati (1996) the increase in the number of mitochondria and lipidic globules, the differentiation of elaioplasts and vesicles are indicative of high activity in the plasmodial cytoplasm. Galati (1996) opines that net of vesicles observed in the tapetum of *Sagittaria* may function as a cytoskeleton. When the microspores are freed, and the periplasmodium is not yet formed, some electron dense membranous fragments fill the anther locule. According to Galati (1996) the origin of this material may be the product of the degradation of cellular walls of meiocytes and rest of the callose.

2.2 Histochemical studies on angiosperm anther.

During last three decades considerable histochemical information on anther development is available (Heslop-Harrison,
1972; Mascarenhas, 1975; Bhandari and Sharma, 1983; Blackman and Yeung, 1983; Panchaksharappa et al., 1985; Shivanna and Johri, 1985; Noher de Halac, 1990; Sheel and Bhandari, 1990; Hegde et al., 1993; Vijayaraghavan and Sudesh, 1994). The unique features of male gamete formation have been presented vividly by Wilson and Yang (2004) and Scott et al. (2004). But, histochemical information on the anthers of hydrophilous plants is almost lacking.

Sporogenous cells are thin-walled and rich in RNA and total proteins (Hegde et al., 1993). They generally lack storage carbohydrates except in *Euphorbia* (Rudramuniyappa and Annigeri, 1985). *Calanthe* (Hegde and Rudramuniyappa, 1986) and *Spathoglottis* (Hegde et al., 2000). In *Citrus* (Rudramuniyppa and Hegde, 1985) sporogenous cells possess lipids. In *Datura* peak activities of non specific esterases, glucose-6-phosphatase, alkaline and acid phosphatases, peroxidase, malate dehydrogenase, succinic dehydrogenase and cytochrome oxidase are recorded in the sporogenous cells (Hegde and Andrade, 1982; Andrade and Hegde, 1983; Hegde, 1985). In the sporogenous cells of *Helianthus* also activities of these enzymes in sporogenous cells are reported by Hegde and Isaacs (1992).
Young meiocytes structurally and histochemically resemble sporogenous cells (Vijayaraghavan and Cheema, 1978; Vijayaraghavan and Sudesh, 1994). In sunflower meiocytes (Smart et al., 1994) biogenesis of mitochondria and in Solanum meiocytes (Bhandari and Sharma, 1988) and Carica meiocytes (Sheel and Bhandari, 1990) presence of granular inclusions are reported. In Spathoglottis meiocytes possess thick PAS-positive walls and storage carbohydrates (Hegde et al., 2000).

Synthesis of callose, inside the primary walls of meiocytes, is a common feature in the angiosperm anthers. In many plants callose is PAS-positive (Bhandari and Sharma, 1983; Katti et al., 1994; Vijayaraghavan and Sudesh, 1994). In Cannabis (Heslop-Harrison, 1964), Chenopodium (De Fossard, 1969) and Iphigenia (Panchaksharappa and Syamasundar, 1974) callose is PAS-negative. Exceptionally, in Spathoglottis callose deposition is partial and occurs only on the outer face of meiocyte aggregate (Hegde et al., 2000). Total absence of callose deposition is reported in Pergularia (Vijayaraghavan and Shukla, 1977). Certophyllum (Takahashi, 1995) and many submarine hydrophilous flowering plants (Ducker et al.,
1978, Pettitt, 1981). In those plants where callose deposition is observed, it persists till the completion of meiosis and formation of microspores tetrads. Latter the callose disintegrates and frees microspores from tetrad condition.

Meiocytes undergo meiosis when they are callose-bound. In *Allium tuberosum* and *Cyclamen persicum* (Bhandari et al., 1981) and *Allium sativum* (Gori, 1983) primary wall of meiocytes persists during meiosis. During meiosis meiocytes exchange their cytoplasm through cytoplasmic channels (Scott et al., 2004; Wilson and Yang, 2004).

Reduction in RNA and protein contents of meiocytes, prior to meiosis, appears to be a general phenomenon in angiosperm anthers (Pacini et al., 1985). However, such reduction is not observed in the meiocytes of *Spathoglottis* (Hegde et al., 2000). At ultrastructure level also meiocytes undergo reorganization. There are changes in the structure of endoplasmic reticulum and their transformation into vesicles (Blackmore and Barnes, 1988), differentiation of plastids and mitochondria (Heslop-Harrison, 1971; Dickinson, 1982) and disintegration of cytoplasmic nucleoloids (Sato et al. 1991). During meiosis, meiocytes are inter connected with plasmadesmata through
the pores present in the callose (Dickinson, 1992). In Beta vulgaris plasmodesmata disappear at prophase-I (Hallden et al., 1991). In sunflower an association between mitochondria and nucleus is observed (Smart et al., 1994). McCormick (1993) reports the synthesis of few meiosis-specific proteins. According to Scott et al. (2004) DNA synthesis and expression of number of nuclear genes decline during meiosis.

Completion of meiosis and the formation of microspore tetrads is marked by the regain in the synthesis of RNA and proteins (Mandaron et al., 1990). 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 microspores.

Microspore tetrad phase terminates when the callose wall lyses enzymatically through tapetally secreted callase (Scott et al., 2004). In Typha, tetrads cohere as a result of fusion of the tectum on the inner faces between neighbouring pollen grains (Takahashi and Sohma, 1984). Tetrads of Pyrola are surrounded by a continuous tectum (Takahashi and Sohma, 1980).
In many plants formation of acetolysis resistant tapetal membrane is reported. In *Sorghum bicolor* all the orbicules fuse to form an orbicular wall on the loculus surface (Christensen *et al.*, 1972). Spropollenin deposition on the inner surface of tapetal cells is also reported in *Simmondsia chinensis* (Chaudhry and Vijayaraghavan, 1995). In tomato, the orbicules remain on the surface of the degenerating tapetum and forms a continuous boundary (Polowick and Sawhney, 1993).

Circumstantial evidences indicate the possible involvement of callose in exine formation as a source of glucose or as a stress factor – compressing and flattening the upper ends of the probuculae to form tacti (Vijayaraghavan and Shukla, 1977). Callose wall acts as a framework and provides a template or mold for exine wall (Waterkeyn and Beinfait, 1970, 1971; Chaudhry and Vijayaraghavan 1996). In Epacridaceae only fragmentary exine is formed at the sites where callose deposition is less (Ford, 1971). In *Pergularia daemia* (Vijayaraghavan and Shukla, 1977), in which callose is absent, exine formation is very poor. In transgenic tobacco, microspores are released prematurely due to early callose dissolution, and possess poorly developed exine (Worrall *et al.*, 1992). In many male sterile
plants premature or persistant callose wall affects pollen formation (Horner and Rogers, 1974; Horner, 1977; Graybosch and Palmer, 1987; Hegde and Isaacs 1992; Agadi and Hegde, 2003). In $Spathoglottis$ $plicata$ exine formation is noticed only where previously callose deposition had occurred (Hegde et al., 2000).

There are also reports on the association of other factors with exine formation. In $Poinciana$ $gillissii$ (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 by the mosaic differentiation of plasma membrane. In $Arabidopsis$ $thaliana$, grown in space-flight conditions, sterile empty microspores possess well-developed normal-looking exine despite the weak deposition of callose and early degeneration of tapetum (Kuang et al., 1995).

Young microspores come in direct contact with the tapetally derived sporopollenin which undergoes polymerization resulting in the formation of autolysis resistant exine wall (Chaudhry and Vijayaraghavan, 1996). Polymerization of sporopollenin involves activity of various enzymes (Sawhney and Bhadula, 1988). The development of free microspores includes the enlargement of spores,
accumulation of reserve substances in them, elaboration of exine and pollen mitosis. According to Reznickova and Willemse, (1980), in *Lilium*, increase in the microspore volume is due to water uptake, whereas according to Moss and Heslop-Harrison (1967), in *Zea mays*, it is due to the removal of constraint imposed by callose wall. Presence of rich quantities of carbohydrates, RNA, total proteins, and ascorbic acid in the old microspores indicates that the increase in the volume of microspores is accompanied by the increase in cytoplasmic contents.

Presence of carbohydrates, RNA and total proteins is a general feature of pollen grains (Hegde *et al.*, 1993; Vijayaraghavan and Sudesh, 1994). During the last phase of its development pollen loses water content and becomes coated with tryphine and pollenkitt which are the degenerating products of tapetum (Pacini, 1992).

Cellular interaction between tapetum and reproductive cells is an indicative of existence of growth correlation between the two tissues. Cell ablation experiments have shown that pollen development will be abnormal in the absence of tapetal tissue (Koltunow *et al.*, 1990). Generally the tapetal cells are non-vacuolate and densely cytoplasmic (Panchaksharappa *et al.*, 1985; Chapman,
1987; Shah et al., 1991; Agadi and Hegde, 2003). Cell walls of tapetum are extremely thin and contain very little cellulose (Bedinger, 1992; Loukides et al., 1995). Exceptionally the tapetal cells in Spathoglottis possess thick cellulosic wall (Hegde et al., 2000). EM studies have revealed the simple, fibrillar walls of the tapetum in tomato (Polowick and Sawhney, 1993). At the time of callose deposition and meiosis, the walls fibrils loosen and appear fibrous with granular inclusions. According to Polowick and Sawhney (1993) the changes in the wall nature of the tapetal cells facilitate the movement of materials into the locule.

During meiosis, tapetum attains peak metabolic activity as evidenced by the presence of RNA, proteins (Hegde et al., 2000; Agadi and Hegde, 2003) and also DNA, histones and ascorbic acid (Bhandari et al., 1976; Chewrot and Gorska-Brylass, 1981; Sheel and Bhandari, 1990). Upto meiotic stage, proplastids present in tapetal cells resemble those in reproductive cells (Paccini et al., 1992). Later, as in Lolium perenne, tapetal proplastids undergo a different program of development from those present in the reproductive cells (Paccini et al., 1992). The microspore proplastids differentiate into amyloplasts whereas in the tapetal cells proplastids differentiate into
Elaioplasts (Polowick and Sawhney, 1990; Paccini et al., 1992). Elaioplasts are specialized type of chromoplasts whose degeneration coincides with the accumulation of lipids.

Tapetum during meiosis is richly populated with 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). Peak activities of non-specific esterases and acid phosphatase (Vithanage and Knox, 1979) and also adenosine triphosphatase, glucose-6-phosphatase, cytochrome oxidase, succinic dehydrogenase and malate dehydrogenase are also recorded in the tapetum (Hegde and Andrade, 1982; Andrade and Hegde, 1983; Hegde and Isaacs, 1992). The secretory function of tapetum at the time of meiosis is self evident by its metabolic hyperactivity. It is unequivocally claimed that tapetum is a transient, nutritive tissue (Chapman, 1987; Regan and Moffatt, 1990; Shah et al., 1991). Tapetum contributes to the regulation of microspore development (Mariani et al., 1990; van der Meer et al., 1992), microspore nutrition (Paccini and Franchi, 1983) and the synthesis and secretion of various lipophilic substances such as pollenkitt and tryphine (Weber, 1992). According to Bhandari and
Khosla (1995), at least in triticale, tapetum is directly or indirectly involved in the synthesis of callose also. Chapman (1987) is of the opinion that the plasmodesmata present between tapetal and sporogenous cells provide the route for translocation of metabolites from storage tissues to the sporogenous tissue.

Tapetum constitutes a major source for the supply of exine materials to pollen grains. Sporopollenin bodies have been associated chiefly with secretory tapetum. Sporopollenin bodies in plasmodial tapetum have been reported in *Butomus umbellatus* (Fernando and Cass, 1994). When pollen grain is 3-celled, periplasmodium in *Sagitteria* disintegrates and part of it is deposited on the exine as tryphine (Galati, 1996).

Once the microspores are released from the tetrads, the tapetal cells start supplying nutrients and components of exine (Pacini et al., 1985). In *Prosopis juliflora* (Vijayaraghavan and Chaudhry, 1993; Chaudhry and Vijayaraghavan, 1996), concurrent with primexine formation, tapetal cells show extrusion of pro-orbicular bodies coated with sporopollenin. According to Heslop-Harrison and Dickenson (1969) and Shoup *et al.* (1980) tapetal cells contribute sporopollenin precursors to the final assembly and/or modification.
of exine. According to Barnes and Blackmore (1988) endoplasmic reticulum in tapetal cells function in the production of sporopollenin precursors. On the contrary, incorporation of tapetally derived orbicules in the exine has been doubted by Shivanna and Johri (1985) and Polowick and Sawhney (1993). Chaudhry and Vijayaraghavan (1996) opine that initial sporopollenin deposition on the microspore wall is provided by the microspore protoplast.

Tapetum, apart from contributing to the exine development, also provides various other metabolites (Mascarenhas, 1975; Bhandari, 1984; Chapman, 1987). During post-meiotic stages of anther development, tapetal cells undergo gradual disintegration. In *Ledebouria socialis* cytoplasmic degeneration of the tapetal cells occurs after pollen mitosis (Hess and Hesse, 1994). After callose dissolution tapetal cells show intense exocytosis of polysaccharides into the anther locule. Thus, the disintegrated callose and/or the tapetal walls are not the only source of highly concentrated sugar fractions of the locular fluid (Hess and Hesse, 1994). In *Arabidopsis* anther a monosaccharide transporter At STP2 facilitates the uptake of hexoses released from degraded callose (Schneidereit et al., 2003). Subsequently the tapetal cells acquire abundant endoplasmic
reticulum in association with osmiophilic bodies, the osmiophilic bodies contain pollenkitt precursor which are flavonoids in nature (Hess and Hesse, 1994).

Occurrence of rich contents of RNA and total proteins, in addition to large quantity of mitochondria, is a characteristic feature of tapetal cells (Moss and Heslop-Harrison, 1967; Lee and Warmke, 1979; Chapman, 1987; Chaudhry and Vijayaraghavan, 1995; Agadi and Hegde, 2003). In *Vigna unguiculata* (Guerra and Carvalheiro, 1994) and *Phaseolus* (Carvalheiro and Guerra, 1994) tapetal cells contain polytene chromosomes. Tapetum is source of β1-3 glucanase (callase) which dissolves callose wall and releases haploid microspores from tetrads (Hird *et al.*, 1993). In *Carica papaya*, PAS-positive material present in the tapetal cytoplasm seems to move in to the anther locule and fills it completely (Sheel and Bhandari, 1990). Presumably these polysaccharides are source for starch synthesis which stores later in the pollen grains.

In many plants, during pre-meiotic phase, anther contains starch grains in the connective and/or anther wall layers (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; Katti et al., 1994; Hegde et al., 2000). Thus, the connective and wall layers serve as sink organs and they represent potential source of carbohydrates for microsporogenesis.

During post-meiotic stages, carbohydrate storage present in the connective and wall layers depletes. In most of the anthers middle layer (s) is ephemeral. Generally endothecium develops fibrous thickenings. Fibrous thickenings are composed of lignin and suberin (Eames, 1961; Freudenstein, 1991). In *Chenopodium rubrum* endothelial fibrous thickenings lack lignin and suberin and contain cellulose (De Fossard, 1969). In *Acacia* presence of callose is reported on the radial walls of endothecium (Kenrick and Knox, 1979).

The anther dehiscence program begins with the degeneration of the middle layer and tapetum, expansion of endothecial layer, the deposition of fibrous bands in endothecium and connective cells. These are followed by degeneration of septum making anther a bilocular structure. Finally there will be a rupture of the stomium.
According to Goldberg et al. (1993) anther dehiscence requires a functional stomium region and dehiscence does not depend on the contents of anther locule (Koltunow et al., 1990; Mariani et al., 1990; Goldberg et al., 1993).

Just before dehiscence and concomitant with lyses of stomium cells, endothecial and epidermal cells become turgid. This generates an inwardly directed force in the anther wall that causes the rupture of the weakened stomium. Subsequent desiccation of the endothecium causes differential shrinkage of thickened and unthickened regions of the cell wall, creating an outward bending force that leads to the retraction of the anther wall and full opening of the stomium to permit pollen release (Scott et al., 2004).

Involvement of Jasmonic acid (JA) and ethylene signalling is also envisaged in anther dehiscence (Rieu et al., 2003). In the anther of JA deficient mutants, because of delay in desiccation, endothecium and connective cells remain fully expanded and locules are filled with liquid.