CHAPTER – V

DISCUSSION
DISCUSSION

Multicellular organisms originate from a single progenitor cell. During the course of growth and differentiation specific loci are activated in response to positional information supplied by cytoplasmic inclusions or by the surrounding tissues (Haig, 1990). The positional information may be provided by a simple gradient, by separate cytoplasmic determinants for different structures, or in the absence of determinants one path of differentiation may act as a "default option" which might change the developmental response to the determinants (Haig, 1990).

In sexual reproductive system the order of events spanning male gametogenesis, female gametogenesis and embryogenesis is strictly maintained (Koltunow, 1993). This is related to substrate-product relationship in which the early event produces a component or a structure essential for a subsequent event (Koltunow, 1993). Generally these sequential events are not disrupted because each event is dependent solely on the completion of the previous event. Alternatively, according to Hartwell and Weinert (1989) feedback controls may exist that keep the
earlier event under surveillance in such a way that if a particular event is not completed, a signal is sent to an active controlling system or checkpoint that acts directly or indirectly to delay or block the initiation of a later event. In this way, checkpoints could actively enforce a dependency and maintain a rigid sequential order.

In flowering plants embryogenesis takes place in both sexual and apomictic developmental pathways. The embryo develops within the haploid female gametophyte surrounded by diploid maternal tissues. Somatic cells are also able to deviate from their normal fate and embark upon an embryo pathway in the absence of a maternal environment (Martienssen, 1998). In flowering plants zygotic embryogenesis involves two fertilization events. One sperm fuses with the egg cell to produce zygote and initiate embryogenesis. The second sperm unites with a secondary nucleus (formed by the fusion of two polar nuclei within the embryo sac or central cell) to initiate the differentiation of the endosperm. Endosperm in flowering plants is a triploid tissue and according to Goldberg et al. (1994), it is neither gametophytic nor sporophytic in origin.
The endosperm develops along with the embryo and provides nutrients for either the embryo, the germinating seedlings or both. Fertilization causes the ovule, containing the embryo and endosperm, to develop into a seed and the ovary to differentiate into a fruit. Fruit facilitates seed dispersal.

Thus, the production of seeds, through sexual reproduction, requires the formation of fertile male and female gametes, pollination, fertilization and embryogenesis. Since the zygotic embryo is surrounded by gametophytic and sporophytic tissues, the coordinated interplay is expected between maternal and gametophytic tissues in the formation of fully differentiated and developed embryo within the seed. In tissue culture such interplay can be mimicked by "nurse cells" that take over the role of maternal tissues (McCabe et al., 1997; Mordhorst, et al., 1997; Russinova and de Vries, 2000).

The questions usually addressed in the examination of seed formation include (i) are the individual ovules fertilized? (ii) If so, are the ovules potentially capable of maturing or is the genetic constitution of the embryo
and or endosperm, simply invariable? (iii) Is the number of ovules that
develop within the fruit limited by resources? (iv) Are there criteria by
which ovules are selectively eliminated? To understand abortion pattern of
the seed, the factors controlling the embryo abortion must be understood.
Therefore, the objectives of this study are to determine whether the seedless
condition is due to (1) defective pollen, (2) defective female gametophyte,
(3) failure of fertilization or (4) defective embryogenesis and endosperm
development.

Although the descriptions of female gametophytes, and embryo and
endosperm development in seeded and seedless grape varieties are
numerous and comprehensive (Nitsch et al., 1960; Barritt, 1970; Pratt,
1971; Kassemeyer and Staudt, 1982; Okamoto and Imai, 1982; Staudt and
Kassemeyer, 1984; Vallade et al., 1987; Ledbetter and Ramming, 1989;
Ebadi et al., 1996), our knowledge regarding the histochemistry of
reproductive structure of Vitis vinifera is very scanty (Considine and Knox,
1979). This is a serious lacuna because light microscopic histochemical
studies help in ascertaining the possible causative factors responsible for
seedless condition. Histochemical studies also help in understanding the structure and function of the different parts of the developing reproductive structures and their interaction with one another.

**Microsporogenesis and gametogenesis**

The review of literature on male sterility reveals that the breakdown of anther development may happen at pre-meiotic, meiotic or post-meiotic phases (Kaul, 1988). The effect of male sterility is expressed either in the reproductive cells or tapetum or both (Hedge and Isaacs, 1992). Absence of callose deposition, delayed or premature callose degradation, failure of spore wall formation, premature or delayed degeneration of tapetum are some of the commonly reported symptoms of the male sterile anthers (Hegde and Isaacs, 1992).

During microsporogenesis and gametogenesis in Thompson seedless grape no aberrant development of anther tissues is observed. The homogeneous mass of cells of anther primordium is characterized by the presence of rich contents of RNA, total proteins and very thin PAS-positive
cellulosic walls. During subsequent growth of the anther primordium, the putative archesporial cells in the hypodermal layer at the four corners become densely cytoplasmic. The enlargement of these cells is accompanied by increase in RNA and total proteins.

The primary wall of sporogenous cells is thin and PAS-positive and cellulose-positive. Presence of rich contents of RNA, total proteins and ascorbic acid, but not storage carbohydrates, in the sporogenous cells is a common feature in many plants (Hegde et al., 1993). Free ribosomes present in the sporogenous cells are responsible for dense basophilic cytoplasm of these cells (Hegde et al., 1993). Presence of other metabolites in the sporogenous cells, such as lipids in Citrus (Rudramuniyappa and Hegde, 1985) and peak activities of several enzymes in Datura (Hegde and Andrade, 1982; Andrade and Hegde, 1983; Hegde, 1985) and Helianthus (Hegde and Isaacs, 1992) suggest that these cells are equipped with suitable metabolic machinery needed for future growth and differentiation. According to Vijayaraghavan and Sudesh (1994) lack of storage carbohydrates in the sporogenous cells is an indication of their utilization to
provide energy and metabolic substances for the differentiating meiocytes.

Meiocytes, histochemically and ultrastructurally resemble sporogenous cells (Vijayaraghavan and Cheema, 1978; Vijayaraghavan and Sudesh, 1994; present study). Active mitochondrial biogenesis in the sunflower meiocytes (Smart et al., 1994) and presence of granular inclusions, presumed to be mitochondria and plastids in Solanum nigrum (Bhandari and Sharma, 1988) and Carica papaya meiocytes (Sheel and Bhandari, 1990), reflect the metabolic potential of these cells. Increase in the granular inclusions during meiotic stages has been linked with sporogametic transition.

Differentiation of meiocytes is accompanied by the synthesis of a callosic wall, inside their primary wall. The changes in calcium ion concentration at plasmamembrane induces callose (β, 1-3 glucan) synthesis from cellulose (β, 1-4 glucan) (Worrall et al., 1992). As in other plants (Panchaksharappa and Rudramuniyappa, 1974; Nanda and Gupta, 1974; Bhandari and Sharma, 1983; Sheel and Bhandari, 1990; Vijayaraghavan and
Significance of spatial and temporal synthesis of callosic wall is not fully understood. It is implicated that callosic wall is essential to isolate the gametophyte progenitor from somatic differentiation signals emanating from the surrounding sporophytic cells (Bouman, 1984; Knox, 1984; Bell, 1992; Koltunow, 1993). Such isolation might also require to prevent the diffusion of harmones or other compounds present in the surrounding wall layers (Carman et al., 1991). Since it functions as a selective semipermeable membrane (prevents the transport of high molecular weight substances), callosic wall possibly creates a different but congenial chemical environment for meiocytes to undergo meiosis (Heslop-Harrison and Mackenzie, 1967; Knox and Heslop-Harrison, 1970; Southworth, 1971). However, occurrence of meiosis in the absence of callose in *Pergularia daemia* (Vijayaraghavan and Shukla, 1977), submarine hydrophyllous flowering plants (Ducker et al., 1978; Pettitt 1981), transgenic tobacco
(Worrall et al., 1992), Ceratophyllum (Takahashi, 1995) and Spathoglottis plicata (Hegde et al., 2000) cast doubt on the role of callose in the induction of reduction division. Moreover reports indicate the apparent ability of some high molecular weight substances to cross the callosic barrier (Mascarenhas, 1975; Rodriguez-Garcia and Majewska-Sawka, 1992). Callose has been attributed with other functions also, such as, isolation of the products of meiosis and prevention of cell cohesion and fusion (Waterkeyn, 1962; Heslop-Harrison and Mackenzie, 1967; Theis and Robbelien, 1990; Bedinger, 1992), protection of meiocytes from dehydration (Barskaya and Balina, 1971) and maintenance of proper functioning of various enzymes involved in the development of primexine and exine and prevention of random oxidation and autopolymerization of sporopollenin precursors (Vijayaraghavan and Shukla, 1977). According to Blackmore and Barnes (1988) the deposition of callose signifies the importance of cytoskeleton in establishing the microspore polarity.

Concurrent to the differentiation of sporogenous tissue, anther wall layers also differentiate. There are four anther wall layers, including an
epidermis, an endothecium, a middle layer, and a tapetum. Tapetal layer becomes metabolically very active during meiosis and degenerates at vacuolate microspore stage. Because of its location and chemical components, tapetal cells are expected to have a cellular interaction with reproductive cells and play a key role in microsporogenesis. Cell ablation experiments have shown that in the absence of tapetal cells normal pollens are not produced (Koltunow et al., 1990). The tapetal cells are non-vacuolate and densely cytoplasmic (Panchaksharappa et al., 1985; Chapman, 1987; Shah et al., 1991; Katti et al., 1994; present study). Tapetal cell walls contain extremely negligible deposition of cellulose (Bedinger, 1992; Katti et al., 1994; Loukides et al., 1995; present study). Ultrastructural studies have shown that tapetal cell walls in tomato are simple, fibrillar and include distinct middle lamellae (Polowick and Sawhney, 1993). During the period of callose deposition and meiosis the tapetal wall fibrils loosen and appear fibrous with granular inclusions, which, according to Polowick and Sawhney (1993) facilitate the movement of material through the wall and into the locule.
At meiosis, tapetum becomes very rich in nucleic acids, proteins, histones and ascorbic acid (Bhandari et al., 1976; Chewrot and Gorska-Bryllass, 1981; Sheel and Bhandari, 1990; Katti et al., 1994; Hegde et al., 2000; present study). In Thompson seedless grape the cytoplasm of tapetal cells stain strongly for polysaccharides. This layer is also rich in 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). Prior to meiosis, both reproductive and tapetal cells contain proplastids (Pacini et al., 1992). But after meiosis, the microspore plastids differentiate into amyloplasts whereas in tapetal cells proplastids differentiates into elaioplasts which are specialized type of chloroplasts whose degeneration coincides with the accumulation of lipids (Polowick and Sawhney, 1990; Pacini et al., 1992). In addition, tapetum shows peak activities of several enzymes (Vithanage and Knox, 1979; Hegde and Andrade, 1982; Andrade and Hegde, 1983; Hegde and Isaacs, 1992). Thus, the tapetum acquires a metabolic hyperactivity at the time of meiosis and functions as a secretory tissue. It is unequivocally claimed that the tapetum is a transient nutritive
According to Chapman (1987) translocation of metabolites from storage tissues to the sporogenous tissue occurs via plasmodesmata present between tapetal cells and sporogenous cells.

Production of healthy and metabolically rich sporogenous cells is also dependent upon the participation of other accessory somatic diploid cells. At late sporogenous stage, anther contains starch storage in the middle and endothecial layers (Reznickova, 1978; Cheng et al., 1979; Reznickova and Willemse, 1980; Bhandari and Khosla, 1982; Bhandari and Sharma, 1983; Andrade and Hegde, 1983; Vijayaraghavan et al., 1987; Sheel and Bhandari, 1990; Hegde and Isaacs, 1992; Katti et al., 1994). The absence of starch storage in the wall layers (present study) indicates its degradation and suggests that the formation of a mature fertile male gametophyte depends on the nutrient supply by the anther sporophytic tissues. This reflects the nutritional correlation between two cell types. Ammonia precursors of lipids, carotenoids and sporopollenin are presumed to be derived from the breakdown product of starch (Atkinson et al., 1972; Reznickova and...
Willemse, 1980). Utilization of carbohydrates in the synthesis of other metabolites is also implicated (Andrade and Hegde, 1983; Vijayaraghavan et al., 1987). According to Mapson et al. (1954) and Chinoy et al. (1967, 1971) carbohydrate pool is utilized in the biosynthesis of ascorbic acid. Ascorbic acid, as a powerful reducing agent (Zholkevich et al., 1965 Burlakova, 1967; Chinoy, 1969), is associated with many significant metabolic processes related to growth and differentiation such as biological oxidation-reduction system (Chinoy, 1962, 1964), cell elongation (Reid, 1937, 1941) and endogenous regulation of auxins and gibberellins (Michniewicz, 1961; Chinoy et al., 1967). In the present study also a correlation is observed between the absence of starch storage in the wall layers and accumulation of ascorbic acid in the connective. Carbohydrate deficiency in the anther causes abnormal pollen development (Sawhney and Bhadula, 1988; Bhadula and Sawhney, 1989; Sawhney, 1992) and it often correlates with male sterility (Banga et al., 1984). As in Theaceae members (Tsou, 1997) tannin begin to deposit in the connective cells at free spore stage and remain until anthesis (present study).
In Thompson seedless grape, meiocytes undergo normal meiosis and cytokinesis when they are still encased within the callose. At the time of meiosis decrease in the stainability for RNA and proteins are observed in the meiocytes, which is a general phenomenon in the angiosperm anthers (Panchaksharappa et al., 1985). The fall in RNA concentration is attributed to the dilution of cytoplasmic content resulting by the enlargement of the meiocyte cells (Moss and Heslop-Harrison, 1967), to the elimination and/or breakdown of ribosomes (Knox et al., 1971) and to the presence of specific nuclease (Dickinson, 1992). It is opined that elimination of cytoplasmic RNA helps in the reorganization of meiocyte cytoplasm so as to eliminate sporophytic influence and to create suitable environment for gametophytic expression (Bird et al., 1983; Rodkiewicz et al., 1986; Dickinson, 1987). The cytoplasmic reorganization also includes changes in the endoplasmic reticulum (Blackmore and Barnes, 1988). The rounding of endoplasmic reticulum into vesicles has been viewed as a mechanism to transmit a sample of sporophytic cytoplasm into the gametophytic generation (Dickinson and Heslop-Harrison, 1977). Sato et al. (1991) report that, only during meiosis, meiocytes show disintegration of cytoplasmic nucleolides
formed by the aggregation of DNA transcripts. After disintegration of nucleolides meiocytes are repopulated with ribosomes.

In some genera, such as *Allium tuberosum*, *Cyclamen persicum* (Bhandari *et al.*, 1981) and *Allium sativum* (Gori, 1983) the primary wall of meiocytes persists during meiosis. According to Chang and Neuffer (1989), the meiocyte wall plays a significant role in the meiotic event as it forms the compact framework that remains unaltered during meiosis. As a result, a large space is provided for the completion of meiosis without outside disturbance. In Thompson seedless grape (present study) primary wall of meiocyte is not distinct. Therefore, it appears that persistence of primary wall of meiocytes is not an indispensable requirement for meiosis to occur (Horner and Rogers, 1974).

Completion of meiosis and the formation of microspore tetrads are accompanied 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. Microspore tetrads lack storage carbohydrates and ascorbic acid (present study).

Microspore tetrad phase terminates when the callosic deposition disintegrates enzymatically by tapetally secreted callase (Hird et al., 1993). As implicated in Petunia, callase remains inactive until the pH of the anther locule drops down (Izhar and Frankel, 1971). In transgenic tobacco microspores are held in tetrads by cellulosic wall and microspore release requires cellulase (Worrall et al., 1992).

The development of free microspores involves the enlargement of spores, gradual accumulation of reserve substances in them, elaboration of exine, pollen mitosis and formation of two celled pollen grains at the time of anther dehiscence. According to Reznickova and Willemse (1980), in Lilium, increase in the microspore volume results from the water uptake, whereas according to Moss and Heslop-Harrison (1967), in Zea mays, it is
due to the removal of restraining influence of callosic wall. Presence of rich quantities of carbohydrates, RNA, total proteins and ascorbic acid in the old microspores (present study) indicates that the increase in the volume of microspores is accompanied by the increase in the cytoplasmic contents.

Accumulation of carbohydrates in the pollen grains is characteristic feature of angiosperm plants (Hegde et al., 1993). Starch granules serve as reserve metabolite during pollen germination. According to Mandaron et al. (1990), in Zea mays, protein synthesis is low during starch accumulation. However, just before the anther dehiscence, several basic polypeptides are synthesized which perhaps require for pollen germination.

The tetrad period, although the briefest during the pollen grain maturation process, is the most complex with regard to the formation of pollen wall (Fernandez and Rodriguez-Garcia, 1988; Chaudhary and Vijayaraghavan, 1996). Pollen wall synthesis involves both gametophytic and sporophytic cells. The two major phases of exine ontogeny are template formation, which takes place when microspores are in callose bound tetrads,
and sporopollenine deposition, which takes place in free microspores.

The primexine formation begins soon after meiosis is completed and is patterned while the microspores are still invested within the callosic wall (Kronestedt-Robards and Rowley, 1989; McCormic, 1993; Vijayaraghavan and Sudesh, 1994; Katti et al., 1994; Chaudhary and Vijayaraghavan, 1995, 1996; present study). It is generally held that the callosic wall acts as a framework and provides a template or mold for exine wall formation (Waterkeyn and Beinfait, 1970; Chaudhary and Vijayaraghavan, 1996). In the absence of callosic wall microspores either lack pollen wall or have abnormally developed pollen wall (Worrall et al., 1992; Hegde et al., 2000).

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 not determined by callose but by the mosaic differentiation of the plasmamembrane. Primexine serves as matrix into which probaculae are first formed and over and under which protectum and exine are formed (Takahashi and Skavarla, 1991).
Young microspores come in direct contact with the tapetal-derived sporopollenin, which by polymerization produces acetolysis-resistant exine wall (Chaudhary and Vijayaraghavan, 1996). The polymerization of sporopollenin from its precursors (carotenes and carotenoid esters) and its subsequent incorporation into exine involve activities of various enzymes (Sawhney and Bhadula, 1988). Esterases are known to be present in the tapetum before its degeneration, and on the microspores at the time of exine deposition (Sawhney and Nave, 1986). In *Prosopis juliflora* (Chaudhary and Vijayaraghavan, 1996) sporopollenin deposition, synthesized by the microspore protoplast at tetrad period within the callose wall, is later continued by the deposition of sporopollenin synthesized by the tapetal cells at young spore period. According to Galati (1996) the part of the disintegrated tapetum is deposited on the exine as tryphine. Thus, principal structural features of the exine pattern are established within the callosic wall by the late tetrad stage and edification and uniform electron density of the extexine is established when the microspores are released from the tetrads (Chaudhary and Vijayaraghavan, 1996).
Tapetum is considered to play a vital role in pollen development by providing variety of metabolites (Mascarenhas, 1975; Bhandari, 1984; Chapman, 1987). The disintegration of tapetum begins during the development of free microspores. In Thompson seedless grape (present study) tapetum possesses rich contents of RNA, total proteins and cytoplasmic polysaccharides. The secretory nature of the tapetum is evidenced by the loss of its cellulosic primary wall, increase in the number of mitochondria and synthesis of RNA and total proteins (Moss and Heslop-Harrison, 1967; Lee and Warmke, 1979; Chapman, 1987; Chaudhary and Vijayaraghavan, 1995). In *Vigna unguiculata* (Guerra and Carvalheira, 1994) and *Phaseolus* (Carvalheira and Guerra, 1994) tapetal cells contain polytene chromosomes suggesting increase in DNA amount through endoreduplication cycles. Thus, the tapetal cells go through a period of hyperactivity before they finally degenerate (Kronestedt-Robards and Rowley, 1989). In *Carica papaya* PAS-positive material present in the tapetal cytoplasm seems to move into the anther locule and fills it, embedding the pollen grains into it (Sheel and Bhandari, 1990). This substance is lost by the anthers when pollen grains become starch rich.
According to Sheel and Bhandari (1990) this locular PAS-positive material is used up in the synthesis of storage starch grains in pollen grains. In the present study also it is observed that degenerating tapetal cells lose their cytoplasmic contents. The degradation of tapetal contents correlates with accumulation of reserve substances in the pollen grains (Pacini et al., 1992; present study).

In Thompson seedless grape anthers, like as in many other angiosperm plants, the expansion of endothecium results in the crushing of the middle layer (Tsou, 1997; present study). The differentiation of endothecial thickenings begins only after the tapetal degeneration is initiated (present study). It is presumed that the healthy tapetum releases an inhibitor product that prevents endothecial development (De Fossard, 1969; Chauhan, 1977). The production of inhibiting substance ceases after the degeneration of the tapetum. The disappearance of starch grains in the endothecium concurrent to the differentiation of fibrous thickenings suggests their utilization in the formation of endothecial thickenings (Bhandari and Khosla, 1982; Sheel and Bhandari, 1990).
The process leading to the anther dehiscence is the result of the program involving all the different types of cells in the anther, some of these processes start very early in anther-pollen development (Pacini, 1992). The mature anther, at the time of dehiscence, contains bicelled, starch engorged pollen grains, well-formed endothecium and epidermis. During the last phase of pollen development, water content of whole anther decreases. Before dehydration is completed, tryphine (Pacini, 1992) and pollenkitt (Keijzer, 1987), the products of tapetum degeneration, are deposited on the pollen surface. Tryphine and pollenkitt serve several purposes, two of which are to cause clumping of the pollen grains and to facilitate their adhesion to pollinators. As a consequence of anther dehydration pollen grains become dormant.

Thus, microsporogenesis and microgametogenesis studies demonstrate that seedless condition in Thompson cultivar is not due to defective pollen, as also reported in partial-female-sterile soybean (Pereira et al., 1997).
**Megasporogenesis and gametogenesis:**

Megasporogenesis and gametogenesis lead to the formation of the embryo sac. This life cycle from megaspore to megagametophyte with the megagamete (egg) takes place in, and is strongly related to, the mother plant-the sporophyte. For viable seed production megagametophyte ensures that it receives nutrients by maintaining the contact with the mother plant, prepares itself to accept the pollen tube, followed by the double fertilization and equips itself for the formation of the embryo and endosperm. These events occur gradually during the life cycle in different stages in cooperation with the mother plant.

In many plants the flower, fruit and seed abortion are traced to the defects in female gametophyte. As per the reports (Pratt, 1971) the ovules in cultivated grapes may abort before meiosis, at 2-nucleate embryo sac stage, or at organized embryo sac stage and these may be accompanied by lack or abnormal development of nucellus, integuments or even due to incomplete anatropy. In 'Sultana', "Monukka" and 'Sultanina' seedless grapes abnormalities are detected in the integuments at the time of anthesis.
(Pearson, 1932). In some seedless grape varieties abnormalities in ovules are detected in (1) megaspore mother cell (2) megaspore (3) coenocytic embryo sac (4) degenerating embryo sac (5) non-functional ovule (6) endosperm (7) nucellus and (8) collapsed ovule (Barritt, 1970). In many cultivars of *Vitis vinifera* Pratt and Einset (1961), Carraro *et al.* (1979), Kassemeyer and Staudt (1982) and Ebadi *et al.* (1996) report ovule without embryo sac or with degenerating nucellus. Abnormal ovules showing lack of embryo sac or empty embryo sac or aberrant embryo sac without nucellus have been reported in other plants also such as *Quercus* (Mogensen, 1975), *Oxalis* (Guth and Weller, 1986), *Manihot* (Ogburia and Adachi, 1995), *Epilobium* (Seavey and Carter, 1996), and *Narcissus* (Sage *et al.*, 1999).

Megasporogenesis in Thompson seedless cultivar ovules exhibits normal *Polygonum* type of development. The hypodermal archespore cell is rich in cytoplasmic RNA and total proteins and its nucleus becomes more voluminous (Bhandari *et al.*, 1980; Hegde and Rudramuniyappa, 1985; present study). Enlargement of archespore cell is accompanied by the increase of the plasma and its organelles such as ribosomes (Willemse,
1992). These suggest the potential of the archespore cell for further growth and differentiation. At this stage, inner integuments differentiate at the flanks of the nucellus. Young integuments are rich in RNA and proteins. In the funiculus the vascular strand differentiates through which the young ovule receives nutrition from the maternal tissues. The absence of starch storage suggests that the reserve carbohydrates are utilized for the growth of the ovule (Israel and Sagawa, 1964, 1965).

In Thompson seedless grape, archespore cell undergoes periclinal division, the inner daughter cell differentiates into megaspore mother cell. Megaspore mother cell enlarges before the initiation of meiotic division. It is characterized by dense cytoplasmic content, rich in RNA and proteins (present study). The occurrence of plasmodesmata connections with the neighbouring nucellar cells is implicated as a feature suggestive of a role in transfer of metabolites (Rangan and Rangaswamy, 1999). The outer parietal cell derived from the periclinal division of archespore cell and epidermal nucellar cell undergoes several periclinal divisions to produce nucellar calotte. The cells of nucellar calotte are distinct from the rest of the nucellar
cells in having relatively more RNA and proteins (present study). Because of the formation of the calotte, the megaspore mother cell is deeply situated in the nucellus.

Although the factors leading to meiosis are still unknown, this phenomenon is associated with several visible changes occurring in megaspore mother cell. Appearance of vacuole like structures in the nucleus and enlargement of perinuclear membrane at zygotene are presumed to be an indication of a renewed interaction between nucleus and cytoplasm (Willemse, 1992). According to Willemse (1992), the random organization of microtubular system in the megaspore mother cell during meiotic induction and the enlargement of the only chalazal end of the megaspore mother cell suggest the establishment of a cell polarity. Later, the plastids and mitochondria are localized near the cell poles with a high number in the chalazal pole. Willemse (1992) reports persistence of plasmodesmata at chalazal side of the megaspore mother cell.
In addition to the above changes, during the transition stage to haploidy, callosic wall deposits around the megaspore mother cell. Callosic wall first appears at the micropylar side and extends in chalazal direction. In Thompson seedless grape, callose wall is thin at lateral side and dense at chalazal end (present study). It is implicated that by way of callosic wall formation around megaspore mother cell cytoplasmic local barrier to high molecular products is formed and partial cell isolation from surrounding sporophytic cells is realized (Willemse, 1992; Koltunow, 1993). The reason for physical isolation of the gametophytic progenitor cell at this stage is to protect megaspore mother cell from somatic differentiation signals (Wylle et al., 1985). Alternately, according to Carman et al. (1991), callose may isolate progenitor from hormones or other compounds in the nucellus that might otherwise diffuse in and disrupt meiosis. Lack of callose around megaspore mother cell of aposporous species suggests that callose deposition is essential for meiosis and therefore in the development of embryo sac (Koltunow, 1993).
According to previous reports, but not in Thompson seedless, another significant change that megaspore mother cell undergoes during meiosis is reduction in the ribosomes and proteins. It is interesting to note that similar reduction in RNA and proteins is observed in anther meiocytes prior to meiosis. Therefore, the reduction in RNA and proteins in megaspore mother cell during meiosis might reflect the removal of diploid gene information in the cytoplasm of the megaspore mother cell. After the first meiotic division megaspore mother cell shows more cytoplasm at the chalazal end. Such asymmetrical distribution of cytoplasm presumably determines the position of functional megaspore.

After the second meiotic division cellularization results in a linear tetrad with four megaspores, rich in RNA and proteins, and are separated from one other by callosic walls (Present study). In Crepis tectorum (Kapil and Bhatnagar, 1981) and Triticum (Leblanc et al., 1995) the cross walls between megaspores are formed by the deposition of fibrillar material. In contrast to meiocytes, only one of the megaspores has the developmental capacity to form megagametophyte. In Thompson seedless
grapes, which conform to Polygonum type, the chalazal megaspore becomes functional megaspore while the other three degenerate. The basis for selection of functional megaspore may be genetical, as in Oenothera (Willemse, 1992), Datura (Haig, 1990) and Glycine max (Kennell and Horner, 1985a). The role of hormones is also implicated in the selection of functional megaspore (Willemse, 1992). According to Kapil and Bhatnagar (1981) the selection has nutritional basis and the functional megaspore occupies a nutritionally more advantageous position in the ovule. Koltunow (1993) reports that non-functional megaspores, but not functional megaspore, are often surrounded by callosic wall. This callosic wall isolates non-functional megaspores from maternal nutrition supplies, whereas the functional megaspore receives optimum nutrient supply due to absence or short-lived callosic wall around it (Haig, 1990). According to Koltunow (1993) the principal function of callose during megasporogenesis appears to isolate and suppress the further development of non-functional megaspores, thus ensuring the participation of only functional megaspore in megagametogenesis. It is reported that all four megaspores would continue to develop if they are supplied with nutrients (Haig, 1986). However, the
circumstantial evidences in Thompson seedless grape do not support the hypothesis of nutrient determination of selection of functional megaspore. Because in Thompson seedless grape all four megaspores are surrounded by callosic wall (present study). It means that, as presumed by Koltunow (1993), in Thompson seedless grape all four megaspores experience similar physiological environment, at least during early stages of development. But the functional megaspore contains more RNA, total proteins and ascorbic acid (present study) and in some species more plastids and mitochondria than non-functional megaspores (Haig, 1990). As already pointed out that the differential distribution of cytoplasmic contents presumably owes to the cell polarity of the megaspore mother cell. Therefore, it seems that the megaspore, which inherits maximum cytoplasmic contents from the megaspore mother cell is destined to become functional megaspore.

During megagametogenesis three phases can be distinguished: the first one is the formation of the coenocyte with the nuclear divisions, the second is the cellularization of the coenocyte and the last phase is the
differentiation of the individual cells.

In many plants, prior to the enlargement, the functional megaspore gets starch and lipids as storage products and the number of ribosomes increases (Willemse, 1992). However, starch storage is not observed in the functional megaspore of Thompson seedless grape (present study). It is suggested that, for its development, the functional megaspore uses the breakdown products of the three degenerating micropylar megaspores, inclusive the callosic wall (Willemse, 1992). The functional megaspore enlarges by vacuolation. After the first mitosis and the following mitoses the central vacuole enlarges. The central nucleus divides and the two daughter nuclei become positioned each at a micropylar and chalazal pole of the coenocyte. The positioning of the nuclei is probably determined by the mitosis and expressed in the position of the spindle. Directed nuclear movements is probably determined, in addition to vacuolation, by the filamentous cytoskeleton (Willemse 1992). In the mature coenocyte four nuclei are positioned at micropylar pole and four nuclei at chalazal pole.
During the formation of the coenocyte, the cytoplasm acquires rich contents of RNA, proteins and ascorbic acid (Tilton and Lersten, 1981; present study). Absence of starch grains in the central cell is reported in several plants (Tsou, 1997; present study). However, at fertilization, occurrence of starch grains in the central cell has been reported in *Franklinia* and *Schima* (Tsou, 1997) and mutant soybean (Pereira *et al.*, 1997). Equipping the embryo sac with such histochemical composition seems to be a determining factor for cellularization differentiation of the coenocyte. In Thompson seedless grape a correlation is observed between depletion in ascorbic acid and the cellularization of the coenocyte at both the micropylar and chalazal pole (Present study). From the three micropylar cells the egg apparatus and from the three chalazal cells the antipodals are formed. Two nuclei, present in the central cell constitute polar nuclei.

The egg apparatus consists of two synergids and one egg cell. All the cells make contact with the micropylar part of the embryo sac. Generally the egg cell contains heritable organelles, storage bodies and microtubules (Huang *et al.*, 1990). Willemse (1992) reports that the egg nucleus is firstly
positioned in the upper part of the cell and most organelles are located near the nucleus. Before fertilization the nucleus and the surrounding cytoplasm move to a chalazal position in the cell. Cell organelles will increase in number before fertilization. However, in Thompson seedless grapes the egg cell shows weak staining for carbohydrates, ascorbic acid, RNA and total proteins (present study).

The synergids of Thompson seedless grape lack well defined filiform apparatus (present study). Ultrastructure studies reveal that the synergids are rich in cell organelles (Willemse, 1992). Generally the cell walls around the synergids and egg are incomplete. At the border with the upper part of the central cell the cell walls are very thin or absent (Willemse, 1992). The function of the synereids is to receive the pollen tube. Commonly one of the synergids will degenerate. Along with nucellus or inner integument, synergids also secrete micropylar exudate, which includes a pollen tube attractant.
The central cell is coenocytic. Only one polar nucleus is observed at micropylar pole near the egg apparatus (present study). The position of the secondary nucleus is very important as shown in the mutant soybean where the ovules aborted when the secondary nucleus was far from the egg cell (Pereira, et al., 1997).

The three antipodal cells are ephemeral and in many ovules they degenerate before organization of the egg apparatus (Barritt, 1970; present study). In other plants, antipodals contain abundant cytoplasm rich in storage products such as amylum, lipid and proteins, which are used after fertilization (Willemse, 1992). The antipodals function mainly in the transfer of nutrition via the symplast to the embryo sac. In the cell wall bordering the central cell the presence of plasmodesmata supports this presumption.

Traditionally, the ovule development has been viewed as a hormonious process in which all the ovular tissues cooperate to produce mature female gametophyte. As it is suggested earlier that the breakdown
products of the callosic wall and degenerated megaspores are reused by the developing functional megaspore. The nucellus, which originates from and develops in close contact with the placental tissue, also assists female gametophyte in nutrition. The correlation between the cellularization of the coenocyte and reduction in the storage carbohydrates of nucellus implies that cellularization is an energy-required process (Miki-Hirosige, 1964; Tilton and Lester, 1981; Wittich, 1996). Since the nucellus envelops the embryo sac and the vascular supply to ovule terminates at the base of nucellus, the growing cells get this nutrition via symplastic and apoplastic transport. The nucellar cells which are adjacent to the embryo sac often develop wall ingrowths, suggesting that they serve as a storage tissue and also as passage for nutrients into embryo sac (Rangan and Rangaswamy, 1999). Although starch storage is not observed in the nucellus of Thompson seedless grape (present study) the increasing accumulation of ascorbic acid in the nucellar cells, especially in nucellar calotte, suggests the utilization of carbohydrates in the biosynthesis of ascorbic acid (Hegde et al., 1993). In some Lauraceae members the elongating embryo sacs cause the degeneration of the nucellar epidermis and become extra nucellar (Heo et
al., 1998). Since the nucellus is the source of pollen tube attractant, degeneration of nucellus causes proembryo abortion and fruit abnormality (Tilton, 1980). Rich mitochondria, wall intrusions, detachment of cuticle and dissolution of middle lamellae in the cells of micropylar nucellus in *Beta vulgaris* suggest secretory nature of the nucellus (Bruun and Olesen, 1989).

It is implicated that the hypostase, besides being a storage tissue during its early stages of development (Rangan and Rangaswamy, 1999), limits extension of embryo sac in the chalazal region (Bouman, 1984), establishes connection with the funicle vascular supply and in turn stabilizes water balance, and facilitates nutrient transport into embryo sac during transformation of ovule into seed (Willemse, 1992). In the present study also rich accumulation of ascorbic acid is observed in the hypostase. Autoradiographic studies by Chamberlin *et al.* (1993) suggest that hypostase may serve as a secondary pathway for solute transport. However, the autoradiographic studies by Coe (1954) and submicroscopic studies by Belyaeva (1983) negate the implication of hypostase in nutrient transport.
The thick walled tannin-filled cells, phenol containing substances or suberin cell walls, the absence of plasmodesmata and rough endoplasmic reticulum do not suggest a role for hypostase in nutrient transport (Belyaeva, 1983). According to Wittich (1996) the hypostase hinders the direct apoplastic transport of sucrose to the chalazal part of embryo sac.

The translocation of nutrients from nucellus embryo sac is aided by the endothelium (inner epidermis of inner integument). In legumes, endothelial cells are glandular and possess rich RNA and proteins (Hegde and Raibagi 1989). In *Linum* starch grains occur in endothelium (Dhar and Vijayaraghavan, 1980). In *Helianthus* Newcomb (1973) reports plasmodesmata between endothelial cells. Rybechenko (1963) has assigned a nutritive role to this tissue because of its ability to accumulate reserve substances. This tissue also possesses secretory properties (Zinger, 1958; Hegde and Rudramuniyappa, 1985). But in *Foeniculum* endothelium is short lived and without any specialized features (Agarwal and Gupta, 1976). In *Calendula*, due to its cuticular nature, endothelium is regarded as a barrier tissue preventing the transport of nutrients into embryo sac (Plisko,
1971). Histochemical constitution of inner integuments in Thompson seedless grape ovule supports the contention of Plisko (1971). In the present study a correlation is observed between disappearance of ascorbic acid, present along the walls, and lignification of cells of inner integument. Therefore it appears that the storage substances present in the integument are rather used for the lignification of their cells. Thus, it is implicated that, not being glandular in nature, the endothelium in Thompson seedless grape has a limited role in the nutrition of female gametophyte (present study).

The present histochemical study on the ovule development in Thompson seedless grape reflects certain unusual features. Firstly, during meiosis, RNA and protein contents of megaspore mother cell remain high suggesting there may not be a complete removal of sporophytic influence at the time of transition to gametophytic generation. Secondly, due to the persistence of callosic wall around all the four megaspores, functional megaspore is not in nutritionally advantageous position. Thirdly, the lignified cell walls of the integuments might hinder adequate flow of nutrients into the female gametophyte. The absence of rich accumulation of
RNA and proteins in the egg reflects its poor metabolic potential. Because the rich presence of histchemical substances in the egg (Jensen 1965, Hegde and Rudramaniyappa, 1985) along with abundant cell organelles (Schulz and Jensen, 1968; Mogensen, 1972, 1973) seem to be essential to ensure sufficient safeguard for the nurture of future sporophyte. Interestingly only one polar nucleus is evident near the egg apparatus.

**Pollination and fertilization**

In many flowering plants, especially in tropical tree species, shortage of pollinators and inadequate transfer of compatible pollen are responsible for seed abortion (Bawa and Webb, 1984). Male reproductive success is often limited by the ability of the male gametes to gain access to female gametes whereas the female reproductive success is limited by their ability to provide resources for eggs and embryos (Williams, 1975; Janzen, 1977). The common occurrence of partly developed seeds in Thompson seedless grape suggests that seed failure in this cultivar is due to post-zygotic abortion and not due to inadequate levels of pollination and stigmatic or stylar incompatibility (present study).
Thompson seedless grape is a self-pollinating species. According to Sage et al. (1999) self-pollinated plants, in comparison with cross-pollinated ones, manifest self-sterility. It has been suggested that, as in *Narcissus*, self-pollen tubes do not provide the appropriate signals for stimulation of ovule and seed development (Sage et al., 1999). There may be a failure of normal growth of ovarian tissues after the entry of a self-pollen tube into the ovule because pollen tube-ovarian interactions are important for stimulation of normal seed development (O'Neil, 1997). Pollen tubes also provide essential stimuli for initiation of the secretory phase of transmitting tissue (Herrero and Arbeloa, 1989), induction of prolonged embryo sac viability (Herrero and Gascon, 1987) and stimulation of carpellary wall development (Fuller and Leopold, 1975). According to Stephenson (1981) and O'Neil (1997), production of many growth regulators depends on post-pollination stimulation events by pollen tubes. In turn, ovules also appear to provide signals through micropylar secretions controlling pollen tube growth and guidance (Cheung, 1996).
The normal development of male and female gametophytes alone will
not ensure the formation of the seed. As advocated by Knox and Singh
(1987), pollen quantity, quality, pollen germination and pollen tube growth
play a major role in seed formation. The success of fertilization depends
upon differential gene expression in pistil and pollen and effectiveness of
the gametes. On the female side genes relating to the regulation of stigma
receptivity, self-incompatibility and ovule viability are vital. On the male
side certain pollen-specific genes, which effect male transmission at
fertilization, are important. The development of pistil into berry is
suggestive that there is successful fertilization of at least one of the four
ovules in Thompson seedless, otherwise pistils abort (Ebadi et al., 1996).

The stigma plays very important role in the process of fertilization
because the angiosperm pollen lands on the stigma. During the final phase
of pollen development the water content of the whole anther decreases.
Before dehydration is complete pollenkitt, the product of tapetum
degeneration, is deposited on the pollen surface (Keijzer, 1987; Pacini,
1992). Tryphine is deposited on the pollen surface earlier, at the microspore
stage (Pacini, 1990). Tryphine and pollenkitt cause clumping of the pollen grains and help in their adhesion to pollinators. The structural and physiological characters of the stigma enable pollen capture, hydration and germination. Stigma also plays a vital role in controlling interspecific hybridization and in regulating compatibility relationships within the species. Like other seeded cultivars of grape, the stigma of Thompson seedless is of wet type. It comprises many multicellular papillae. Studies have revealed that stigmatic exudate, which stains metachromatic red with toluidine blue, stains positively with PAS and Ruthenium Red but not with protein stains, is secreted from underlying cells of the transmitting tissue (Considine and Knox, 1979). According to Considine and Knox (1979) the extracellular mucilage is continuous from the loculi of the ovary to the stigma surface thus providing considerable volume of secretion required at anthesis. After pollination, stigma withers. Recent report reveals the presence of a arabinogalactan protien in the pistil which functions in recognition, adhesion, pollen tube nutrition and pollen tube guidance (Majewska-Sawka and Nothnagel, 2000).
According to Heslop-Harrison (1992) wet stigmas are associated only with gametophytic self-incompatibility systems, where the pollen genotype itself determines rejection or acceptance. The rejection reaction usually occurs in the style. In Thompson seedless grape good percentage of pollen grains germinate and grow. For pollen germination and growth sucrose, boric acid, calcium, zinc, manganese and iron are required and presumably the exudates of the stigma contain these. Between the hydration and germination, many morphological and biochemical changes, such as protein synthesis, take place in pollen grains. The rough endoplasmic reticulum become free in the cytoplasm of activated pollen grains and dictyosomes start producing vesicles (Cresti and Tiezzi, 1992). The growth of the pollen tube takes place only in the tip region. It is now well established that calcium plays an important role during pollen germination and pollen tube tip growth (Cresti and Tiezzi, 1992). Both membrane associated and free cytoplasmic calcium show gradient distribution from tip to base of the actively growing pollen tubes (Steer and Steer, 1989). EM studies have shown that in many species a characteristic functional zonation exists along the length of the pollen tube. The growing zone is restricted at
the tip and contains vesicles, a sub-apical zone rich in organelles, particularly golgi producing vesicles, a wide nuclear zone containing vegetative nucleus and generative cell or sperm cell and vacuolization and callose plug formation zone dividing the living part of the tube from the inactive ones. The active part of the pollen tube exhibits an intense streaming of cytoplasm. Many investigators have revealed that fertilization in angiosperm relies on directed tip growth of pollen tubes through the style to reach the embryo sac. Immunolocalization studies have shown that in *Nicotiana tabacum* (Li et al., 1995), *Lilium longiflorum* (Jauh and Lord, 1996) and *Nicotiana alata* (Ferguson et al., 1999) the cell wall behind the tip of the pollen tube contains arabinogalactan proteins, which are involved in tip growth of the pollen tubes. Growing pollen tubes in the style produce and secrete cell wall degrading enzymes, which are involved in the breakdown of the intercellular substance of the transmitting tissue.

A correlation between pollen tube competition and abortion of developing seeds or fruits has been recognized (Mulcahy, 1979; Stephenson, 1981; Willson and Burley, 1983). The higher abortion rate
among ovules close to the pedicel may result from delayed fertilization by slower growing and possibly genetically inferior pollen tubes (Schaal, 1980; Bawa and Webb, 1984; Guth and Weller, 1986), despite the likelihood that ovules in these positions would receive adequate supplies of energy and nutrients for development.

Before the pollen tubes enter the embryo sac, the ovule enters the activation phase caused by the pollination stimulus in the form of signals (Willemse, 1992). This activation is expressed by the movement of the nuclei of the central cell and egg cell and in the onset of protein synthesis. The pollen tube grows along the surface of the placenta towards the ovule. Then it enters the micropyle, directed chemotropically by substances produced by the ovule secreted through the micropyle. The synergids are considered to produce such chemotropic substances and to excrete them into the filiform apparatus (Van Went, 1992). From the filiform apparatus the substances leach out into the micropyle or nucellus, creating gradient that influences the growth direction of the pollen tube. Thompson seedless grape has crassinucellate ovules and it has been found that more than one pollen
tube reaches the nucellus, but only one pollen tube actually penetrates. Apparently, recognition and blocking system is operating leading to the rejection of super numerous pollen tubes.

Ultrastructural studies have shown that pollen tube enters the embryo sac at the micropylar side, penetrates through the filiform apparatus into one of the synergids, but never enters the egg cell or the central cell (Van Went and Willemse, 1984). Eventually, the filiform apparatus plays a key role in the interaction between ovule and the female gametophyte. Filiform apparatus is largely composed of pectins in which a loosely organized cellulosic microfibrillar network is embedded (Van Went, 1992). Presumably the specific composition and the physical properties of filiform apparatus allow the pollen tube to penetrate by mechanical forces. Another possibility, according to Van Went (1992), is that the pollen tube excretes enzymes which transform the filiform apparatus into a suitable and attractive pathway for pollen tube. But in Thompson seedless grape pollen tubes successfully enter the ovules, inspite of absence of well-defined filiform apparatus in the synergids (present study).
In Thompson seedless grape one of the synergids degenerates at the time of anthesis. According to the literature after the pollen tube has passed the filiform apparatus it enters into one of the synergids, which has already degenerated. Soon after the pollen tube reaches the synergid cytoplasm, its growth ceases and the tube opens injecting a considerable portion of the tube content, including the vegetative nucleus and the two sperm cells. At the time of actual fertilization the male female gametes are transformed in complete or partial naked protoplasts. Plasmamembranes of the sperm cells become directly appressed to the plasmamembranes of the egg cell and the central cell. The degenerative changes in the penetrated synergid are not just result of the pollen tube entrance and the interaction between the pollen tube and synergid content, but they appear to be vital prerequisites for successful fertilization. The nuclear fusion of the sperm and egg and of sperm and central cell starts with local contacts and fusion of subsequently the outer nuclear membranes and the inner nuclear membranes (Mogensen, 1982). Finally the contents of the male and female nuclei intermingle, which marks the completion of fertilization. In Thompson seedless grape, since egg of all the ovules get fertilized, but polar nucleus fails to develop into endosperm,
it is presumed that double fertilization might not have occurred.

**Embryogenesis:**

During embryogenesis in higher plants the specifications of meristems and shoot-root plant body pattern, differentiation of the primary plant tissue types, and formation of a specialized storage organ needed for seed germination and seedling development occur. The new sporophyte remains dormant until conditions are favorable for post-embryonic development (Goldberg *et al.*, 1994). Three general phases, based on distinct developmental and physiological events, are recognizable during plant embryogenesis. They are (i) Post-fertilization proembryo (ii) Globular-heart transition, and (iii) Organ expansion and maturation.

In Thompson seedless grape embryogenesis is arrested at post-fertilization proembryo phase. Post-zygotic seed abortion has been reported in a wide variety of angiosperm species. All seeds or only few seeds in a fruit may abort (Pereira, *et al.*, 1997). Guth and Weller (1986) and Seavey and Carter (1996) are of the opinion that embryo failures, in *Oxalis* and
Epilobium respectively, are the consequences of genetic factors intrinsic to each ovule. Culture studies indicate that all the ovules that develop a normal embryo sac are potential seeds, but first one to be fertilized suppresses the normal development of the others (Nakamura, 1988). It has been hypothesized that early initiated embryos might act as resource sinks and thereby garner a disproportionate amount of maternal resources (Mogensen, 1975; Lee and Bazzaz, 1986; Hossaert and Valero, 1988). In other words a process similar to apical dominance in stems is working in the ovary where the first ovule to be fertilized produces growth regulators that prevent further development of the other ovules in that ovary (Mogensen, 1975). Vascularization of the ovary (Watson and Casper, 1984) also has been projected as explanation for position-dependent ovule fates in the ovary.

According to the hypothesis proposed by Arathi et al. (1996) seed abortion is mediated by an intense intra-fruit sibling rivalry to gain disperse advantage. It is shown that aqueous extract of dominant seeds inhibiting the uptake of nutrients by healthy subordinate seeds thereby causing their
abortion (Arathi et al., 1996; Krishnamurthy et al., 1997). Diffusates from the dominant seeds, which contained IAA and abortion causing functional chemical, presumably a small molecular weight highly diffusible indole compound, also caused the abortion of subordinate seeds. According the general model of seed abortion proposed by Krishnamurthy et al. (1997), in some ovules, due to temporal differences in fertilization, indole compounds are synthesized and such ovules acquire dominance over unfertilized ovules. These dominant ovules inhibit diffusion of auxin from subordinate ovules, which leads to starvation of subordinate ovules due to lack of phloem differentiation in them. Further, the accumulation of indole compounds results in the ethylene synthesis in the subordinate ovules causing their abortion.

In Dalbergia, the intra-pod seed abortion proceed from the base to the tip of the pod, as a result of which, predominantly distal ovules develop into seeds (Ganeshaiah and Uma Shaanker, 1988). According to these authors the dominance of the distal embryos is generated by the initial head start in sink establishment capacity resulting from the early fertilization of the distal
ovules by pollen tubes that are the first to reach the ovary and perhaps represent genetically more fit pollen grains. The abortion of basal embryos is presumably mediated by a chemical produced by the dominant embryos at the tip. It is likely that the concentration gradient of such an aborting chemical is maintained from the tip to the base, leading to systematic abortion. The degenerating ovules, if fertilized, are also capable of development provided the dominance of the distal embryos is removed. Decrease in the average weight of basal seeds also suggests the non-availability of resource materials, which may be an alternate cause for seed abortion (Ganeshiah and Uma Shaanker, 1988).

The resource sink theory and the theory of dominance of first fertilized ovule over the late fertilized ovules are not applicable for the seed abortion in Thompson seedless grape because in this cultivar all the four seeds abort. In Thompson seedless grape berries are stenospermic. The development of embryo does not proceed beyond few-celled proembryo stage. Stenospermic seeds are reported in other seedless varieties of grapes also (Pearson, 1932; Barritt, 1970; Pratt, 1971; Staudt and Kassemeyer,
In Thompson seedless grape zygote is characterized by dense cytoplasm, rich in polysaccharides, RNA, proteins but not ascorbic acid (present study). These cytoplasmic contents are uniformly distributed in zygote. In the zygotes of other genera, such as *Arabidopsis* and *Capsella*, the nucleus and most of the cytoplasm are present in the upper portion of the cell, whereas a large vacuole is present in the middle to lower portion (Goldberg, *et al.*, 1994). Such asymmetric distribution of cellular components seems to be a prerequisite condition to establish polarity within the zygote. Thus, the uniform distribution of cytoplasmic contents in the zygote of Thompson seedless grape marks the first visible expression of abnormality (present study).

The potential of the zygote to initiate sporophyte growth is reflected in the cytoplasmic organization of the egg itself (Raghavan, 1976). In many taxa the growth potential of the egg is revealed by rich presence of RNA, proteins and cell organelles (Jensen, 1965; Schulz and Jensen, 1968; 1984; Ebadi, *et al.*, 1996).
Vijayaraghavan et al., 1972; Mogensen, 1972, 1973; Hegde, 1985; Hegde and Rudramuniyappa, 1985). Since the egg is situated in the embryo sac, the main function of the central cell appears to sustain the biochemical growth of the egg and subsequently that of the zygote (Bhandari et al., 1980). Presence of abundant cell organelles and metabolites in the central cell, synergids and antipodals (Diboll and Larson, 1966; Diboll, 1968; Cass and Jensen, 1970; Vijayaraghavan et al., 1972) and occurrence of plasmadesmata between synergids and egg (Jensen, 1965) support the growth of the unfertilized and fertilized egg. Thus, the egg and the zygote are provided with a congenial physiological environment that ensures sufficient safeguard for the nurture of the young embryo. Presence of carbohydrates, lipids, nucleic acids and proteins in the zygotes of many taxa reveal the self-sufficiency of the zygote and its preparedness for future development.

Contrastingly, less carbohydrates, RNA, proteins and ascorbic acid in the egg and central cell of Thompson seedless grape suggest low metabolic activity. Surprisingly, inspite of poor metabolic potential of the egg, the
zygote of Thompson seedless grape shows presence of rich quantity of cytoplasmic polysaccharides, RNA and proteins (present study). The accumulation of these substances in the zygote may be related to the degeneration of synergids. This correlation implies that the breakdown products of degenerating synergids provide nutrition to the zygote. The central cell may not be assisting the zygote nutritionally since it lacks storage nutrients even after fertilization (present study). This casts doubt about the nutritional role of the central cell although it is necessary for zygotic embryogenesis because it contains the egg and synergids that are required for fertilization and endosperm development (Goldberg et al., 1994). But for embryogenesis the embryo sac is not an absolute necessity because somatic embryos produced from the sporophytic cells and embryos induced to form from microspores develop outside the embryo sac. These suggest that normal embryonic processes can be carried out in the absence of factors produced by either the female gametophyte or maternal sporophytic tissue. This is supported by the fact that in many mutant embryos defects in zygotically acting genes are responsible for altered development of embryos (Goldberg et al., 1994).
In overwhelming majority of the flowering plants the first division of the zygote is transverse and asymmetric (Goldberg et al., 1994). Asymmetric cleavage of the zygote establishes a polarized longitudinal axis within the embryo. Asymmetric division causes asymmetric distribution of cellular components, most of the cytoplasm in the small terminal cell. The large basal cell is characterized by the presence of vacuole. Because of these differences, the developmental responses of terminal and basal cells differ from one another. The small terminal cell gives rise to the embryo proper that will form most of mature embryo. Cell lineages derived from the terminal cell and embryo proper will specify the cotyledons, shoot meristem, hypocotyle region of the embryonic axis and part of the radical. The large basal cell of the zygote will divide and form highly specialized, terminally differentiated embryonic organ- the suspensor. The suspensor anchors the embryo proper to the surrounding embryo sac and ovule tissue and serves as a conduit for nutrient to be passed from the maternal sporophyte into the developing proembryo. Suspensor senesces after the heart stage and is not functional part of the embryo in the mature seed. The derivatives of the uppermost cell of the suspensor (hypophysis) contribute
to the formation of the root meristem (Vallade, et al., 1987). In many cultivated seeded grapes embryogenesis follows similar pattern.

In Thompson seedless grape, unlike in seeded grapes, zygote divides transversely but not asymmetrically. As a result both terminal and basal cells receive identical amount of cellular components (present study). Embryo morphogenesis and cell specification events are directed primarily by the zygotic genome and maternal transcripts do not contribute to the early zygotic embryo (Russinova and de Vries, 2000). After the division of zygote different gene set act in the terminal and basal cell. The polarization of egg cell, the zygote or both, which does not take place in the egg and zygote of Thompson seedless grape, might influence the differential gene expression events early in embryogenesis. It is not known whether already existing regulatory factors within the egg cell or de novo synthesis of regulatory factors that are distributed asymmetrically to the terminal and basal cells of the embryo trigger the specification of the embryonic derivatives (Goldberg et al., 1994). For any reason if terminal and basal cells of the embryo remain unspecified, the subsequent derivatives also
remain undifferentiated.

According to Stephenson (1981) one of the factors that decides the reproductive potential of the plant is the ability of the maternal parent to provide the necessary resources for development. The resources accumulated in plants are allocated to growth, maintenance and reproduction. The resources allocated to reproduction are further divided between the male and female functions. The resources allocated to the female functions are partitioned between fruit/seed number and weight. From this viewpoint it is possible that during most part of their life, until they become fully mature, the proembryos are heterotrophic. Hegde and Raibagi (1989) recognize seed coat independent, seed coat dependent, endosperm dependent and autotrophic phases during embryo development in *Vigna sinensis*. Plasmodesmata connections among the cells of the embryo and between embryo and surrounding tissues suggest interaction between the different parts of the developing seed, especially in relation to translocation of metabolites between them. In many taxa depletion of storage material in the surrounding tissues correlates with proembryo
growth (Newcomb, 1973; Newcomb and Fowke, 1974; Agarwal and Gupta, 1976; Hegde and Panchakshrappa, 1985). According to Ebadi et al. (1976) the progression of the development in seeds indicates a succession of influences from pollination and fertilization to testa and nucellus, to endosperm, and then to the embryo. Therefore it is necessary to pay attention to the structure and function of the endosperm, nucellus and integument throughout seed development.

The comparative anatomical studies between seeded and seedless grapes show that in seeded grapes, after fertilization, shape of seed changes due to intensive meristematic growth in the funiculus, raphe, chalaza and outer integument (Pratt, 1971). Outer integument thickens and elongates to form beak, which is composed of sclereids, and crystal-bearing cells. Middle layers of the outer integument in the basal half of the seed form two projections (seed folds or fossettes) on either side of raphe, which pushes inner integument and nucellus inside. The inner epidermis of outer integument becomes very thick at beak and thins at fossettes. The cells contain lignin (except fossettes). The rest of outer integument is
parenchymatous. The cells of the inner integument divide anticlinally to keep pace with outer integument. The outer epidermis of inner integument has spiral thickenings, inner layers are irregularly thickened and contain tannin. The middle layer is nutritive, becomes depleted and crushed when endosperm grows rapidly (Pratt, 1971). Nucellus grows by cell enlargement and division. At chalaza nucellus is replaced by cellular endosperm. Basal end of seed enlarges.

On the contrary, in stenosperm seeds of Thompson and other seedless varieties of grapes the soft seeds often show abnormal seed coats (Pratt, 1971; present study). Outer integuments do not form fossettes on either side of the raphe. Prior to anthesis entire outer and inner integuments contain lignified cells. Tips of inner integument exceed outer integument even before anthesis. At chalaza the nucellus is not replaced by the cellular endosperm. Presumably, these thick walled cells of the integuments and tannin-filled endothelium inhibit the translocation of assimilate into nucellus and then to embryo sac. Thus, the female gametophyte, at the time of initial development of zygote and proembryo is subject to malnutrition.
Although the presence of continuous cuticular layer occurs in the inner seed coat of seeded grapes (Esplie et al., 1980) and cereals (Oparka and Gates, 1982) it is suggested that the function of the cuticular layer as a diffusion barrier is to control imbibition before seed germination. The very heavy cuticle, which develops in sugar beet ovules after fertilization, is believed to act as a similar barrier for the transport of nutrients and water during seed development (Bruun, 1992).

Growth hormones play important role in the growth and development of fruits and seeds. Nucellus and seed coats are known to produce auxins (Nitsch et al., 1960). The hormones produced by the seeds play a leading role in the mobilization of resources into the developing fruits (Stephenson, 1981; Wiens et al., 1987). The lack of storage carbohydrates and ascorbic acid in the seed coats and nucellus of the seeds of Thompson seedless grape (present study) suggest that there is no production of auxins and as a result the resources fail to accumulate in the seed. This ultimately results in the inadequate supply of nutrients to the proembryo, which in turn, results in the arrest of its growth.
However, Chamov (1979), Westoby and Rice (1982) and Willson and Burley (1983), Haig (1986, 1987, 1990), Queller (1983, 1984) are of the opinion that, because of their conflicting interests, the surrounding tissues may not assist the growth and development of female gametophyte and embryo. Because the maternal tissues and offspring tissues are genetically distinct and their relationship cannot be compared to that between genetically identical tissues. For instance the occurrence of hypostase only in the aborting ovules of *Pisum sativum* is interpreted that this tissue acts as an instrument by which the mother plant imposes abortion on individual offspring (Briggs et al., 1987). The development of hypostase is also observed in seedless grapes (present study) but its role in stenospermy is not ascertained.

According to Goldberg *et al.* (1994), the role of maternal tissue or accessory cells of the female gametophyte in the formation of and subsequent embryonic development is not clear. In contrast to the suggestion that the maternal sporophyte provides physical support structures and nutrients for the embryo, Martienssen (1998) suggests that zygotic
embryogenesis can occur in the absence of surrounding ovular tissues. This presumption gets support from the fact that somatic cells from variety of vegetative and reproductive tissues undergo embryogenesis in culture and lead to the production of fertile plants in similar way zygotic embryo does. In addition, spatial and temporal gene expression programmes appear to be similar in somatic and zygotic embryos. Embryo-like structures leading to plantlets can form directly from the attached leaves of some plants. In vitro fertilized zygotes produced by fertilizing the eggs in vitro undergo embryogenesis in culture and give rise to flower producing plants. Finally, ultrastructural studies suggest that there is barrier between the inner ovule cell layer and the embryo sac that prevents the transfer of maternal resources directly between these compartments.

Endosperm is an important surrounding tissue of the embryo and is regarded as the main source of food (Bhatnagar and Sawhney, 1981). It supplies carbohydrates, fats, oils, proteins, amino acids, vitamins and growth regulators to the developing embryo. Digestion of the endosperm during the embryo development is an evidence for the dependence of
embryo on endosperm. A correlation is observed between endosperm development and growth substances in the berries of concord and concord seedless grapes (Nitsch et al., 1960). A low level of growth substance in the berries was observed when there is little endosperm development and high level of growth substances when there is rapid growth of the endosperm. In many plants endosperm dysfunction results in embryo death (Wiens et al., 1987). But it is important to realize that the nutritional relationship between embryo and endosperm begins at precise stage during embryogenesis (Raghavan, 1976). During early stages of embryogeny, endosperm appears to have a little nutritive value (Newcomb, 1973; Chamberlin et al., 1994; Pereira et al., 1997). In flowering plants, including seeded grapes, endosperm grows rapidly along with the embryo (Kassemeyer and Staudt, 1983; Ledbetter and Ramming, 1989). In such a situation, endosperm itself will require nutritional supply from the surrounding tissues for its own growth (Schulz and Jensen, 1974; Newcomb, 1973; Agarwal and Gupta, 1976).
In seeded grapes the endosperm is helobial type and develops throughout seed development. Therefore, grapevine has an endospermous seed. But in Thompson seedless grape endosperm fails to develop.

The circumstantial evidences indicate that in Thompson seedless grape the endosperm fails to develop due to failure of double fertilization. Serial sections of the ovules showed only one polar nucleus near the egg apparatus. This suggests that another polar nucleus fails to migrate from the chalazal end and as a result secondary nucleus is not formed. At few-celled proembryo stage also no trace of endosperm is found suggesting the failure of double fertilization and therefore failure of endosperm formation. In partial-sterile 1 mutant soybean also the abortion of the proembryo is attributed to the failure of endosperm formation (Pereira et al., 1997). In this mutant it is observed that the ovules with a fused polar nucleus away from the egg aborted and ovules with a fused polar nucleus near the egg became seed (Pereira et al., 1997). The reason for the non-migration of the fused polar nucleus is presumed to be due to failure of central cell vacuole to shrink (Kennel and Horner, 1985b; Folsom and Cass, 1992). In Thompson
seedless ovule also large central vacuole persists which, as suggested by Cass et al. (1985), influences the nuclear position.

Even if double fertilization occurs in some ovules of Thompson seedless grape, the metabolic state of the central cell may become responsible for the non-development of endosperm tissue. According to Ogburia and Adachi (1995), in Manihot, the ill developing embryo and endosperm suppress the ability of embryo sac to absorb nutrients from the nutrient rich surrounding sporophytic tissues. As a result both proembryo and endosperm tissue are subjected to malnutrition condition eventually resulting their degeneration.

In Thompson seedless grape, the proembryo may not be endosperm dependent, but older embryo will be endosperm dependent for its development. Zygote and proembryo may absorb whatever nutrients available from the central cell. Such situation also reported in other plants (Hegde and Raibagi, 1989; Folsom and Cass, 1992; Chamberlin et al., 1994). This explains why the seedless ovules appear normal from the zygote
to the early proembryo stage.

Although evidences suggest that developmental arrest of the embryos is often preceded by the degeneration of the endosperm (Meinke, 1986). In Orchidaceae and Podostemonaceae embryo development takes place in the absence of endosperm. In such cases the embryo draws nutrition directly from the surrounding tissues. In this regard the suspensor acts as a conduit for the metabolites for the developing embryo from the surrounding sporophytic tissues (Vijayaraghavan et al., 1988; Hegde and Raibagi, 1989). The presence of wall ingrowths and occurrence of plasmodesmata between the walls of the suspensor cells indicate that the suspensor helps in absorption of nutrients from the adjacent integumentary cells to the developing embryo. The presence of mitochondria with well developed cristae, a few ribosomes, endoplasmic raticulum and dictyosomes with numerous vesicles near wall ingrowths are the parameters usually associated with wall labyrinths that are believed to be involved in short-distance transport (Vijayaraghavan et al., 1988). This helps in proper embryo development. In many legumes from pre-globular proembryo stage
onwards, the chalazal and middle regions of the suspensor show presence of starch grains (Hegde and Panchaksharappa, 1985; Vijayaraghavan et al., 1988). Thus, the basal region of the suspensor, with well-developed wall labyrinths, participates in the absorption and translocation of metabolites while the chalazal and middle regions act as storage polysaccharides. *Beta vulgaris* ovules, in *In vitro* culture, have shown that during initial period, when the placental nutrient supply is cut off due to excision of the ovule, the rapid metabolism of starch in the suspensor cells keep embryo alive (Bruun, 1992). This supports the contention that the primary function of the suspensor is to orient the embryo in close proximity to the source of nutrients in the ovule or embryo sac (Raghavan, 1980). From this perspective, the failure of differentiation of suspensor in the embryo of Thompson seedless grape contributes to the developmental arrest of the embryo (Present Study).

The contention that the lack of resource material is responsible for the arrest of embryonic growth (Ebadi et al., 1996) gets support from culture studies where the embryo completes its full growth and germinates
into seedling (Emershad et al., 1989). In addition, the physical environment, provided by endosperm, is presumably needed for the activation of genes at each specific stage. Because the development of the embryo, like male and female gametophytes, reflects some underlying sequence of gene expression. Presumably, each stage and each cell type is characterized by the activity of specific set of loci. The genetic control of development consists of switching-on and switching-off of particular sets of loci at appropriate stage of development. Thus, development is seen as a genetically controlled algorithm that produces mature embryo from a zygote (Haig, 1990). The algorithm requires a complex set of "instructions" that are executed in a precise order and this order is specified in the instructions. Presumably, during the development of the embryo such instructions generate from the endosperm. Since, in seedless vines, due to absence of the endosperm such instructions are not executed for the switching-on of needed sites of loci for further development. Alternately, as suggested by Koltunow (1993), the ovule specific sexual reproductive events in angiosperms are executed in a particular order, which is governed by the production of a component or structure by substrate. This component is
essential for the initiation of subsequent event. It would be difficult to disrupt the order or timing of events because each event is dependent solely on the completion of the previous event. For example, callose wall formation is conserved during embryogenesis in both sexual and apomictic developmental pathways in plants (Williams et al., 1984; Wakana and Uemoto, 1987, 1988). This consistent occurrence of callose suggests that it may be necessary for embryo development whether or not the embryo is derived by sexual or asexual processes. In Thompson seedless grape the failure of production of specific component, such as callose, or failure of embryonic structures such as suspensor, due to lack of resources, disrupts the order of development because of non-completion of the previous event. But still, though its growth is arrested at early proembryo stage, the embryo remains viable even in the mature berry. The cultures of these embryos make them to develop further and produce a fully differentiated sporophyte (Emershad et al., 1989). In Thompson seedless grape the underdeveloped embryos retain cytoplasmic polysaccharides, ascorbic acid, RNA and proteins even at mature berry stage. Presumably, through funiculus, the embryo must be receiving some nutrient supply, just enough to keep it
viable throughout.

The evidence presented here supports the view that the developmental arrest of the embryo in the Thompson seedless grape is due to lack of nutrient supply from the surrounding sporophytic tissues. To the malnutrition condition of the central cell contributions come from the abnormal seed coats, failure of differentiation of suspensor and lack of endosperm formation. This results in the lack of specific substrates required to activate specific genes needed for the initiation of subsequent developmental stages.