Discussion
Pre-meiotic:

At premeiotic phase of anther development in both fertile and sterile lines of *Raphanus* and *Pennisetum*, sporogenous cells and PMCs have thin PAS-positive walls and high amounts of cytoplasmic and nucleolar RNA and proteins. AA is also considerable in the cytoplasm. The high concentration of RNA in these tissues coincides with the diffused state of the chromatin, as indicated by a weak feulgen stainability in their nuclei. Further, the large nucleolus usually reflects the physiologically active state of the cells (Avers 1976). Thus, the sporogenous cells and PMCs are metabolically very active. Similar cytochemical features and active metabolic state of these cells have also been reported in a number of plants (see reviews Bhandari and Sharma 1983, Panchaksharappa *et al.*, 1985, Rudramuniyappa and Mahajan 1991; Annigeri 1984,1985; Bhandari 1984; Rudramaniyappa 1991). At the prophase of meiosis, partial decline in the intensity of cytoplasmic RNA in both fertile and sterile lines was noticed. This reduction, with the deposition of callose around meiosis, appears to have corresponding correlation. During meiosis declining trend of RNA becomes a feature, as observed in the cytoplasm of meiocytes both in fertile and sterile lines of *Raphanus* and *Pennisetum*. Therefore, decline in RNA as well as parallel rise in metabolic activity appears to correspond with the callose building around the meiocytes. Decline in RNA in the cytoplasm of meiocytes during prophase is due to reduction in ribosome population. This feature has been amply substantiated (Mackenzie *et al.*, 1967; Heslop-Harrison 1971; Dickinson and Heslop-Harrison 1977), and later a number of cytochemical studies have supported this (Panchaksharappa and Rudramuniyappa 1974; Rudramuniyappa and Panchaksharappa 1980,1982; Rudramuniyappa and Annigeri 1984,1985). In both fertile and sterile lines of *Raphanus* and *Pennisetum*, as in the present study, the development, isolation and insulation of meiocytes by callose is a normal happening. However, it has been reported by Warmke and Overman (1972) that
the central mass of callose fails to be dissolved in CMS Sorghum. Following tetrad formation, the microspores are normally released after the dissolution of callose which is deposited around the tetrads of both Raphanus and Pennisetum. However, the release of microspores from the tetrads is occasionally inhibited in CMS Raphanus. But, as reported, tetrads also degenerate shortly afterwards (Kakihara et al., 1988). In contrast to the result of Bartkowiak-Broda et al. (1970), this finding does not represent the common type of degeneration. Microspores do not be released from the tetrad and also degenerate subsequently, as has also been observed in male sterile lines of Brassica (Cole 1959), Soybean (Graybosch et al., 1984) and Pepper (Horner and Rogers 1974). In the present study degeneration of tetrads was observed wherever the formation of hypertrophiad tapetum occurs. Thus there is a correlation between the hypertrophy of the tapetum and degeneration of tetrads. In all these examples, like the Petunia (Bino 1985) the disturbances appear to begin, as has been recognised here, at the instance of tapetum. The tapetal cells either become vacuolated or additionally proliferated and invade the anther locule. Generally, these abnormalities seem to occur long before microspore degeneration.

In angiosperms two kinds of tapetum have been well recognised: secretary and plasmodial. In the former, tapetum secretes nutrients into the sporangial locule via the locular fluid, whereas in the latter the tapetal cells loose their walls, enlarge and establish a very close physical contact with the developing microspores (Pacini et al., 1985). In the normal anthers, timing of release of nutrients from the tapetum and the absorption of these by the microspores is very well synchronized and obviously is a well correlated happening. This coordinated developmental requirement represents one of the best examples of intertissue relationship in plants. Any disruption in this process leads invariably to pollen abortion commonly referred to as male sterility (Vasil 1967; Laser and Lersten 1972; Mascarenhas 1975; Bhandari 1984; Kaul 1988). Tapetum is known to supply several substances to the developing microspores namely, enzyme callase, carbohydrates, pollen wall materials, enzymes, etc. These are degraded or leached...
as such into the anther locules. A delay in the release of any one or all of these substances could conceivably have an adverse effect on pollen development. In the present study on both *Raphanus* and *Pennisetum*, pollen abortion is a post-meiotic happening. The production and release of enzyme callase from the tapetum is apparently not affected because the callose around the tetrads is normally digested and the microspores are separated. However, at times the microspore tetrads are crushed due to physical enlargement of tapetum as noticed, which is due to persistence of callose or to abnormal enlargement of tapetal cells amounting to later malfunctioning. The present study reveals that the degeneration of tetrads of spores appears to be due to abnormal behaviour of tapetum rather than belated or no release of callase. Further, although the release of microspores is normal, their subsequent growth is arrested. For a brief period, the microspores are surrounded by their own walls formed. This suggests that the production and release of precursor materials for primexine formation from the tapetum is not deficient.

Various kinds of abnormal development of tapetum have also been reported in several other cytoplasmic and genic male sterile lines, (Laser and Lersten 1972; Gottschalk and Kaul 1974; Frankel and Galun 1977; Bhandari 1984) including *Raphanus* and *Pennisetum* of the present study. The author, however, hesitates to suggest here that the genes for male sterility in *Raphanus* and *Pennisetum* probably have operated through tapetum which affects the development of microspores. Several abnormalities in tapetal growth have been reported such as: considerable enlargement and or vacuolation of tapetal cells (Overman and Warmke 1972; Cheng *et al.*, 1979; Lee *et al.*, 1979; Greyson *et al.*, 1980; Bino 1985); small size of tapetal cells (Murthi and Weaver 1974; Stelly and Palmer 1982); premature degeneration of tapetum (Fillion and Christie 1966; Horner and Rogers 1974; Graybosh and Palmer 1985); delayed degeneration and persistence of the tapetum (Chowdhury and Dass 1968; Overman and Warmke 1972); vacuolation and enlargement of tapetal cells in CMS-C corn (Weicheng and Shaofen 1988); finally tapetal enlargement, cellular disorganization and formation
of intratapetal syncytium in Sorghum (Overman and Warmke 1972). Earlier, in Pennisetum itself tapetum is reported to persist some time and becomes highly vacuolate in CMS (Reddy and Reddi 1974). There are a number of instances where tapetal cells show no difference in the cytoplasmic particles, as in the case of wheat (De Vries and Ie 1970); normality in the structure and number of organelles as in CMS-C maize (Lee et al., 1979); absence of obvious visible differences in the organelles of CMS soybean (Albertsen and Palmer 1979) and vacuolation appearances just before any visible defect in microspores as in Brassica (Theis and Robelen 1990). In the present study on microsporogenesis of CMS Raphanus, no difference was observed between sterile and fertile lines until the completion of meiosis. However, when the microspores are just released from the tetrads and when the tapetal cells begin to enlarge, subsequently cellular disorganization of tapetum occurs resulting syncytium. The latter state shows abundant vacuoles in tapetum.

As reported in Triticum male fertile line, starch granules rapidly disappear from the degenerating tapetal cells, whereas in its male sterile line the dissolution of starch is much slower (Joppa et al., 1966). The persistence of the tapetal cells having starch grains has been observed in CMS Sorghum (Singh and Hadley 1961). In the former, while male fertile pollen are filled with starch, pollen in the sterile line have no starch. Similar observation has also been made by Rogers and Edwardson (1952) in CMS corn. The mature pollen of both Raphanus and Pennisetum here are filled with PAS grains. Several such aberrations in the CMS anthers possibly result from a common mechanism such as: disturbance in the balance of phytochromes as described in barley and Triticum (Colhoun and steer 1983; Ahokas 1982; Saini and Aspinall 1982). It appears that there is a common protein deficiency for such aberrations which have been reported in male sterile plants of Petunia and Brassica (Wu and Murray 1985; Banga et al., 1984; Angadi and Anand 1987). Since proteins in the anther can exert enzyme functions, a deviated enzymatic activity is possible due to deficiency of proteins. Esterase in particular is of overall importance during the degeneration of tapetum and also
for the polymerization of sporopollenin (Hohler and Borner 1980; Shawnay and Nave 1986; Shawnay and Bhadula 1988; Vithanage and Knox 1979). Whether such differences in enzyme activity are the cause (Hohler and Borner 1980), as suggested or the cause is only the result of mere degeneration remains to be answered. Since tapetum in *Pennisetum* is persistent in the present observation, it can be assumed that the impairment in esterase activity appears to be a cause, a defect in male sterility. As has been shown in these earlier studies, pollen abortion can occur at any stage in microsporogenesis. Since the tapetum is a major source for a variety of substances needed directly or indirectly for pollen maturation, obvious to infer that dependence on tapetum and any deviation in pollen developmental pattern would inevitably lead to pollen sterility.

**Callose:**

As made known hitherto and as numerous studies have demonstrated in their observations, the pollen sterility may occur at any stage of microsporogenesis (Kaul 1988). It is principally difficult to define exactly the stage at which the abortion is initiated because a wide range of histological disturbances cause varying expressions. There are other kinds of aberrations reported in the literature namely aberrant callose behaviour. At premeiotic stage and degeneration of the tapetum deviated from normal. With the onset of meiosis, each PMC differentiates and undergoes meiosis. In both sterile and fertile lines as observed here in *Raphanus* and *Pennisetum*, additional wall thickenings developed around the meiocytes react negatively to RNA and proteins tests, but show positiveness to PAS reaction. Therefore, it is obvious that the callose wall is necessarily deposited around the PMC.

Early breakdown of microsporogenesis could be explained by aberrant behaviour of callose. But, in the present study, meiosis in both sterile and fertile plants is normal up to microspore tetrad formation. Hence, the callose deposition is a requirement. Whenever callose persists, the PMCs and microspores which are retained within tetrad, as in case of soybean (Warmke and Overmann 1972), result
in the formation of cenocytic microspores. Similar formation was observed occasionally in the present study. But such spores are surrounded by callose in a tetrad. Stelly and Palmer (1982) have also described male sterility with the formation of syncytia due to aberrant callose distribution. These and other reports explain the crucial role of callose during microsporogenesis. Evidently, callose is essential to prevent the fusion of meiocytes until the release of microspores from the tetrad. On the other hand, an excess of callose can also cause male sterility. In the present study no such aberrant behaviour of callose was observed. Due to failure in resorption of callose, which results due to abundance of it encasing the four microspores, degeneration is reported at the tetrad stage itself of the male sterile line (ms Takgi) of Brassica (Thesis and Robelen 1990). Consequently the microspores are not released and therefore, donot become functional. A similar degeneration has also been described by Izhar and Frankel (1977) in male sterile line of Petunia. Further studies on the cause for failure of callose digestion reveals that, in male sterile plants at tetrad stage, pH of the locule remains high in sterile plants as compared with normal fertile plants. As such no activity of the pH, specific to enzyme callase, was detected. Izhar and Frankel concluded that resulting false timing of callase activity seems to be a causative factor for the male sterility.

Callose wall is generally considered as a selective "Molecular Filter" permitting only the passage for basal nutrients, but not for the larger molecules (Heslop-Harrison 1964). This proposal (concept) was later testified variously and found to be correct to some extent. It was verified by the incorporation of radioactive thymidine into PMC's before the deposition of callose as it was not possible after the deposition of callose (Heslop Harrison and Mackenzie 1967). In 1971, Southworth observed the entry of glucose and sodium acetate, but not phenylalanine which consists of smaller molecules than those of glucose. Similar transfer of colloidal iron (Rowley and Dunbar 1970) and tetrazolium salts (Sauter and Maquardt 1970) was also demonstrated. Thus, callose wall is found to act not merely as a molecular filter, but also isolates PMCs from the external environment.
becoming a barrier in a way. However, within the area of callose wall materials deposition, cytomeiotic channels have been reported (Whelan et al., 1974). These channels apparently do not provide biochemical isolation from the tissues surrounding sporophylls particularly, tapetum. The precocious dissolution of callose and alsolete lag in its dissolution have been noticed in a few plants (Daman 1961, Warmke and Overman 1972; Frankel et al., 1969; Izhar and Frankel 1971). However, such a prolonged degeneration of callose is due to faulty timing of enzyme callase activity. In the present study no aberrant behaviour of callose has been noticed, hence the normal meiosis. Tapetum functions normally and completion of meiosis takes place causing timely synthesis of enzyme callase. According of Knox (1984), callose helps to separate the gametophyte from sporophyte. Delay in callose breakdown is possibly due to reduced callase activity (Bino 1985) rather than faulty timing, as suggested by Izhar and Frankel (1971); Warmke and Overman (1972); Nanda and Gupta (1974); and Horner and Rogers (1974). There are also reports wherein callose deposition around the meiocytes and tetrads has not been observed, as reported in Pergularia (Vijayaraghavan and Shukla 1977) and Pandanus (Periasamy and Amalathas 1991). In the former absence of callose is correlated with the poor development of exine. Barring these two reports, occurrence of additional callose wall around meiocytes and tetrads is almost a universal feature. In the present study too, its positive role is in both fertile and sterile anthers is well documented. The sterility which has been confirmed in the present study on the Raphanus and Pennisetum, is therefore, decidedly post-meiotic occurrence at microspores stage. Deposition of callose is obviously normal in its known function of assisting completion of meiosis is PAS-positive nature of callose has also been reported earlier along the callose wall (Rudramuniyappa and Annigeri 1984, 1985). In its deposition, small AA grains are noticed (Rudramuniyappa 1991). The degraded materials of callose in normal healthy anthers are generally utilized by the developing microspores in pollen formation for the nutrition and wall formation for the pollen. In the sterile anthers these products are mixed up with degenerating locular contents.
Post-meiotic:

Formation and separation of microspores was studied and observed in CMS anthers. The process of their formation is similar to that of fertile anthers, which clearly suggests that the processes of meiosis is normal and complete. Deposition of primexine around the microspores was also noticed. Earlier studies regarding absence of or reduction in the deposition of callose in some plants have been correlated with the reduction of primexine deposition, and also lack of normal exine ornamentation (Ford 1971; Sampson, 1977; Vijayaraghavan and Shukla 1977; Mc Gione 1978; Periasamy and Amalathas 1991). Callose is also known to prevent the random oxidation and autopolymerization of sporopollenin precursors, which helps in precise development of pollen wall and exine pattern.

The microspores of fertile line have shown to possess comparatively richer RNA, proteins and ascorbic acid than the sterile one. This physiological state of desparity reflects possible metabolic differences between the sterile and fertile lines at the cellular level. Partially, this difference is confirmed by the ultrastructural details which account for the pollen organization as observed in wheat (Hu et al., 1977). However, it is observed here that the microspores in sterile line degenerate abruptly. This abortion is the result perhaps of disintegration of nucleus and loss of cytoplasmic RNA, AA and proteins. No synthesis of starch is seen in the aborting microspores which is in contrast to the positive situation in the fertile spores in which starch synthesis, not only initiate but also increases as the microspores develop into maturity. Therefore, developing microspores in fertile line are distinctly different in their shape, possessing normal exine and high amounts of RNA, proteins, AA, and also rich starch in the cytoplasm. In some fertile taxa, starch is not at all observed in the mature pollen (Chauhan 1980). Storage of starch during pollen grains development is one of the important features in the reproductive cycle (Pacini et al., 1986). Further, recent studies reveal that not only its occurrence but also physical and chemical features provide useful data in understanding several aspects of pollen biology. It is indicated that the starch grains present in the pollen are of two types: black and
dark-blue when tested with 1KI (Baker and Baker 1979). Recently, Franchi and Pacini (1988) surveyed in detail and found the occurrence of an other kind of insoluble polysaccharides in shedding pollen. They reported not only amylose and amylopectin but also dextrine which stain from dark red to brown with IKI test. Further, it is to be remembered that the absence of starch does not mean that there are no polysaccharides at all. Pollen without starch also stains strongly with PAS test indicating even diffuse spreading of polysaccharides in the cytoplasm. Starch containing and starchless pollen grains may also occur in the same anther (Franchi and Pacini 1988). In the normal development, sugars are absorbed by the microspores or else developing pollen grains may utilize the deposited starch rapidly. In both these cases, starch is used as energy source for the normal metabolism for the building up of pollen gametophyte and its wall.

In fertile anthers of Raphanus and Pennisetum, tapetum shows signs of autolysis and degeneration parallely with the development and differentiation of microspores. The contents of degenerating tapetum are utilized by the developing microspores, thus indicating obvious fulfilment of its functional role in the development of viable pollen. Similar histochemical features and behaviours of normal tapetum have been reported earlier (See Pacini et al., 1985, Panchaksharappa et al., 1985, Rudramuniyappa and Annigeri 1984, 1985; Bhandari and Sharma 1983). All these studies and the present study substantiate the possible functional role of tapetum in the development and differentiation of viable pollen.

Wall layers and connective:

Microsporogenesis in angiosperms is a sequential and orderly process in anther development involving several structural and histo-and bio-chemical changes in the heterogeneous tissues composition of anther. Although, the anther wall layers, endotegu and connective are equally important, and contribute to pollen formation, it is the tapetum that plays a crucial role in providing necessary energy nutrients to the developing microspores and pollen. Anther wall comprises
of epidermis, endothecium and middle wall layer(s). Morphologically, these wall layers are considered to protect the developing microspores, and the endothecial thickenings, differentiated are helpful in anther dehiscence. A number of studies made indicate that anther wall constitutes an accessory tissue for remote nutritional source for developing microspores. Considerable attention has been paid to the role of anther wall layers. Studies made hitherto indicate that these wall layers, apart from their protective role, are known to perform several important physiological functions in establishing nutritional correlation in the differentiation of pollen grains. But, apparently, these wall layers do not play any role in causing male sterility as indicated in the present study on *Raphanus* and *Pennisetum*. In both fertile and sterile plants, storage PAS grains are observed in anthers. However, their occurrence is not a consistent feature. Lipids, are also observed in the wall layers and connective. In wheat, early stages of both male fertile and sterile anthers showed starch accumulation in endothecium (Anonymous 1976). At maturity, the starch storage was lost from the endothecium in these and the fertile pollen is endowed with starch storage. Similarly, in maize (Cheng *et al.*, 1979), endothecium and epidermis at pre-meiotic stages of both sterile and fertile anthers show the deposition starch. In the former, starch is lost following the disorganization of tapetal cells at young microspore stage. Contrary to this, in the fertile anthers, starch accumulation persists even at its maturity. Persistence of starch in the wall layers of normal anthers has been observed earlier (Panchaksharappa *et al.*, 1985). In addition to starch, a number of enzymes namely, phosphatases, oxidases and esterases, have been recorded both in the present study and earlier studies (Hegde and Andrade 1982; Bhatia and Chopra 1978; Georgieva 1978; Georgieva and Tsikova 1977): The reserves namely starch and lipids, are present in both wall layers and the connective, and degrade into soluble sugars which translocate easily into the neighbouring tissues. Thus, these surrounding tissues with their storage and also enzymes, perhaps compensate the timely requirements, when the meioocytes are bracketed with the callose wall and undergo growth and differentiation in the normal development of anthers. The
situation in sterile anthers seems to be different: until meiosis is completed wall layers do perform the role of storage for the supply of needed materials to the central vital tissues. In regard to degeneration of microspores in these, in the absence of a clear evidence, the said role in the supply of needed nutritional materials seems to be doubtful. Histochemical and micro-autoradiographic studies on sugarbeet, corn and Sorghum (Nakashima and Hosaoka 1970, 1971, 1974; Nakashima 1975) have revealed that in these reducing sugars gradually increase in the endothecium and connective of both fertile and sterile anthers. In both these plants, starch storage was observed at anthesis in the tissues of fertile anthers, but not in CMS anthers. These and other studies do provide an evidence of dubious role, the wall layers play in the cause of anther sterility.

Contrary to the fall in the concentration of RNA and proteins, staining intensity for DNA content in the nuclei do not decline in the wall layers of Raphanus and Pennisetum. Ascorbic acid is also found to be considerably high in both sterile and fertile anthers of these plants. These substances are high even in the connective. Wall layers storage, in addition to the supply of nutrients to the developing meiocytes and microspores, is also utilized for the growth of anther wall layers themselves and also for the differentiation of endothecial thickenings. In Raphanus and Pennisetum both fertile and sterile anthers develop endothecial thickenings. However, in Pennisetum, endothecial thickening increases at maturity which contributes to the failure in the dehiscence of anthers as reported by Reddy and Reddi (1974). Almost similar observation has been made in Orchard grass (Fillion and Christie 1966) and Sorghum (Webster and Singh 1964). In Beta maritima (Coe and Stewart 1977) anther dehiscence was prevented because of nondevelopment of endothecial thickenings. Reznickova and Willemsie (1980) stated that any increase or decrease of cellular storage in anther wall layers, would cause starch formation in endothecium and middle layers. Breakdown products of starch constitutes one of the main sources of locular fluid which is utilized as nutrition by the developing microspores and pollen and even in the anther wall formation. Occurrence in anther of lipids and different kinds of
enzymes, particularly in the middle wall layers, is involved in the synthesis of sporopollenin precursors. An intermediate product formed in the locules could be thought of as an active acetate which may constitute a common precursor for the synthesis of lipids, carotenoids and perhaps even sporopollenin (Atkinson et al., 1972). The breakdown products of starch can be related to the formation of such products (Reznickova and Willemsen, 1980). Studies of this kind are to be augmented necessarily for correct understanding of the functional behaviour of the middle wall layers and endothecium in normal development of anthers.

There are two sources for synthesis of sugars which need to be conveyed to the tapetal cells in anther development: First source is from vascular bundles of the anther, which convey nutrition through connective and endothecium. The second one is from the storage starch and lipids of endothecium and middle wall layers. Tapetum in turn stores sugars in the vacuoles and supplies them to the differentiating PMCs or pour into the locules. These sugars are absorbed by the developing PMCs, microspores and pollen. The sugars absorbed by these tissues could be deposited as starch storage or at times transformed into ascorbic acid or may be utilized rapidly. In the present study, the normal pollen is known to store starch as well as AA. Therefore, polysaccharides present in the anther wall layers are utilized as energy source for the gametophytic metabolism, and also as precursors for the formation of callose and pollen walls.

Tapetum also influences the development of endothecial thickenings (Shivanna and Johri, 1985). Absence of endothecial thickenings during anther development is due to the inhibition caused by the tapetal products. Failure of endothecial thickenings in the sterile anthers has been reported in Phaseolus (Pritchard and Hutton, 1972); Belamcanda chinensis (Gupta and Nanda, 1973); Zea mays (Cheng et al., 1979); Cajanus cajan (Dundas et al., 1981) and Gossypium (Singh et al., 1989) and barley (Kaul and Singh, 1966). An increase in thickness and thickenings of endothecium has contributed to the possible failure of anther dehiscence in CMS Tift-23 Pennisetum (Reddy and Reddi, 1974) and Orchard grass.
(Fillion and Christie 1966). However, in CMS *Raphanus* and *Pennisetum* a normal development of endothecium was observed. However, in the former, at maturity, epidermis persists in CMS anther having a number of starch grains suggesting possible unutilized starch which gets stored.

Young connective tissue at early stages of anther development in *Raphanus* and *Pennisetum* has small quantity of PAS - Positive grains as storage. As the anther develops, the storage increases considerably. Often, an increase in the accumulation of PAS grains was observed towards the anther locule. In this area connective also shows storage of AA. Connective normally contains low content of RNA and proteins in the cytoplasm. However, most of the enzymes localized show their minimal activity in this tissue. This area generally receives the nutrients from the adjoining vascular tissue from which distribution of enzymes normally occurs. It is apparent that there is no difference between fertile and sterile anthers in respect of storage and metabolic functions of connective tissue. It has been indicated that the endothecium and vascular strands of the anther are under the influence of male sterile gene which attacks and thereby, influence the pollen abortion (Laser and Lcrsten 1972). In wheat (Joppa et al., 1966) vascular development in male sterile (CMS) anther is poor and therefore, it decreases starch production caused by the tapetal cells. Lack of starch in the developing microspores is correlated to reduced solutes (Sugars) transport into the stamens of sterile plants.

**Histo-Enzymology:**

The biological shape, function and activities of cells and tissues are determined by DNA. Responsible agents are gene products, the enzymes and also the structural proteins. Clear understanding of development and behaviour of multicellular organism is dependent on a detailed knowledge of the mechanisms, and understanding of the regulation of enzyme systems and their activity. Although, some advances have been made concerning the Phenomenon of cytoplasmic male sterility, still much is needed to be understood about its mechanism. In the present study an attempt has been made to understand the
possible involvement of some of the enzymes during pollen abortion in *Raphanus* and *Pennisetum*. In CMS *Raphanus* mitochondrial enzymes namely, malate dehydrogenase, glutamate dehydrogenase, succinate dehydrogenase and isocitrate dehydrogenase show considerably low activity in the syncytium tapetum at the microspores formation when these are just released from tetrad. This enzyme state obviously suggests a low rate of respiratory activity which is generally correlated with low population of mitochondrial presence which in turn, disturbs the respiratory system. This might hamper severally the development of microspores and may even result in sterility.

In *Zea mays*, activity of glucose-6-phosphate dehydrogenase is low in mst anthers (Markova 1981a). Throughout the period of microspore development, malate dehydrogenase is reduced in mst anthers having T.C. or S-cytoplasm maize (Ohmasa et al., 1976). With the advancement in microspore development, enzyme activity decreases in mst anthers. In the mft- and mst- version of maize line; VIR-29, activity of enzyme glucose-6-phosphate dehydrogenase during microsporogenesis is nearly same until the tetrad stage (Danilenko 1981). Thereafter, enzyme activity decreases with the progression of microsporogenesis in male sterile anthers, but, on the contrary increases in male fertile anthers. Initial signs of deviating development of CMS-type of *Petunia* anthers are visible apparently at the first stage of meiosis itself (Bino, 1985 a). The beginning of abnormalities are represented by the presence of large vacuoles in the cytoplasm of the tapetal cells (Bino, 1985 b). Until it reaches early meiosis, microsporogenesis in CMS *Petunia* anthers proceeds normally, but becomes indistinguishable from the situation in male fertile-type anthers, later. Correspondingly, during early developmental stages, the activity of cytochrome oxidase in both male fertile and CMS anthers are similar. However, first differences in total cytochrome oxidase activity was observed in extracts of meiotic and post meiotic stages of microspores. Decrease in activity of CMS-type anthers corresponds with the decline in the total proteins also. Apparently, the differences in cytochrome oxidase activity are consequence of the progress in degeneration
process of tapetal and sporogenous tissues. In *Raphanus* the first sign of
degeneration was observed in the tapetum at the young microspores stage itself.
The meiocytes in CMS anthers show minimal activity of cytochrome oxidase. In
CMS-S and CMS-C (Bino *et al.*, 1986), cytochrome oxidase activity is reduced
from premeiosis onwards in contract to its activities in male fertile anthers. In all
CMS maize systems, the cytochrome oxidase activity changes from premeiosis
onwards, and first symptoms of degeneration is evident and varies from the early
meiotic stage to the binucleate pollen stage. In *Pennisetum*, however cytochrome
oxidase activity persists in the tapetum of CMS anthers and also in fertile anthers
at microspore stage. Activities of these oxidoreductases in CMS anthers have been
the object of intense studies. The nature of CMS in this regard is attributed to
the altered function of organelles, particularly mitochondria, as enzymes are also
closely associated with mitochondria. Therefore, further detailed study in this
regard is very much needed. Long persisting tapetum in the CMS anthers shows
disturbances in the electron transport and consequently affects seriously the
developmental functions. This conclusion is possible if one takes into account the
reduction of succinic dehydrogenase activity as well as cytochrome-C activity in
the tapetum. It is difficult to presume similar disturbances in the electron
transport of persisting tapetum in *Pennisetum*.

In CMS anthers of Tobacco (Georgieva 1978) during tetrad stage of anther
development, tapetum behaves normally in showing rich activity for
oxidoreductases (cytochrome oxidase and succinic dehydrogenase). It is obviously a
well expressed possibility for oxygen consumption. After the tetrad stage which is
marked by hypertrophy, tapetum does not seem to be operating normally but
shows disturbed respiratory chains, because tapetal cells lose the enzyme activity
of the succinic dehydrogenase and cytochrome oxidases. The reduction of
activities of these enzymes in the case of hypertrophy of tapetum in CMS anthers,
very significant and contributes to the anomalous development of pollen grains
that normally leads to their abortion. In *Raphanus* (the present study) CMS
anthers, after tetrad stage, develop syncytium tapetum showing almost similar type
Possibly low activity of these enzymes disturbs the respiratory chain leading to the formation of aborted pollen, hence the male sterility. It is generally known that the enzyme cytochrome oxidase is principally located in the mitochondrial matrix. Its activity is considerably lower in the sterile anthers than those of corresponding mfl anthers of maize (Bino et al., 1986). Its minimal activity normally corresponds to structural alterations in mitochondria. This was observed in T-anthers but no such phenotypically detectable alterations have been observed in S and C anthers of maize. The depressed enzyme activity, in all mst type of maize anthers, may be due to insufficient translocation of photosynthates from rachis to anthers, as reported by Nakashima and Hosokawa (1974 b). This observation by the later suggests disturbances in carbohydrate and phosphate metabolism in mst anthers (Markova 1981 a,b). In these reduced activity of enzymes glucose-6-phosphate dehydrogenase and NAD dependent glutamate dehydrogenase (GDH) was found, as compared to the mfl anthers. Such a reduction of enzyme activity was also observed in Sorghum vulgare (Alam and Sandal 1969) in which low activity of cytochrome oxidase was reported in addition. This suggests inefficient oxidative phosphorylations which might induce or cause pollen abortion in mst anthers. Low activity of estrases at later stages of anther development has been reported in CMS wheat (Hohler and Borner 1980) and Petunia (Van Marrewijk et al., 1986). In mutant tomato (Sawhney and Bhadula 1988), low esterase activity was implicated for delayed tapetal degeneration and also absence of exine deposition. In CMS Pennisetum too, this kind of observation was made which strengthens its role in bringing not only tapetal degeneration but also its involvement in the synthesis of precursor materials of exine.

The role of peroxidases in plant tissues is immense. Physiologically peroxidases are of interest because of their involvement in numerous catalytic activities. They have the ability to oxidize IAA (Stonier et al., 1979), and hydroxylation of proline (Ridge and Osborne 1970), biosynthesis of lignin (Siegel 1953, Fielding and Hall 1978) and also wall formation (De jong 1966). In CMS
anthers of *Raphanus* and *Pennisetum*, a considerable reduced activity of peroxidase in the wall layers and tapetum was observed resulting in the lack of exine formation in the abortive spores. Any physiological changes in tapetum which in all probability brings malfunctioning of the tissue, is due to structural alteration. This has been considerably explained in *Cucumis melo* (Chauhan and Singh 1968) wherein the low activity of acid phosphatase is observed in the tapetum. In a number of malfunctioning tumor tissues, presence of large quantity of acid phosphatase has been generally noticed. Comparative account on the activities of non-specific esterases and acid phosphatase activity in the tapetum of CMS and fertile anthers of Tobacco has been given (Georgieva and Tsiikova 1977). During tetrad stage, there is a high level of acid phosphatase activity in the tapetal cells of all sterile and fertile anthers. At the stage of microgametogenesis, the acid phosphatase is active in the tapetum, only in those CMS forms in which no hypertrophy of the tapetum was possible. In these cases it may be assumed that in CMS anthers, the hydrolytic process regulated by acid phosphatase and esterases are not disturbed. On the other hand, in case of hypertrophy of the tapetum in the CMS anthers, the esterase activity of the tapetal cells does not change as compared with the normal tapetum. However, activity of acid phosphatase is much lower, since it participates not only in the hydrolysis of sugar phosphates, but also in the metabolism of sugars (see Georgieva and Tsiikova 1977) possibly, if it takes part in the elaboration of nutrients for the use of pollen and reduced activity of the hypertrophied tapetum of CMS line. Thus the process of transport of assimilates from the tapetum is disturbed. (Georgieva and Tsiikova 1977). In *Raphanus* after tetrad stage in CMS anthers, syncytium tapetum comparatively shows low activity of the enzyme- acid phosphatase when compared to the fertile line. This indicates that the processes of transport of assimilates from the tapetum to the microspores seems to be disturbed.

Esterases are synthesized in the tapetal cells (Knox et al. 1973). In *Brassica oleracea* esterase activity is continuous and it accumulates in the tapetum until the tissue degenerates. At this stage, pollen wall esterase activity increases reflecting the
transfer from tapetal cells to the exine cavities (Vithanage and Knox 1976). The present study on *Pennisetum* corroborates with the view of Vithanage and Knox (1976) regarding normal anther esterases activity which is high in the tapetum. This reflects on the transfer of esterases to exine of pollen. In *Petunia* (Van Marrewijk et al., 1986) male fertile anthers, esterase activity is concentrated in the outer tapetal layer till the early microspore stage. But, in male sterile anthers, esterase accumulation in the tapetal cells stops at the moment when the tapetal cells breakdown becomes visible. This feature suggests that differences in esterase activity and the tissue composition are the results of an effect rather than a cause of pollen abortion. In barley (Ahokas 1976), the esterases are responsible for the yellow staining of the sporogenous cells, and are probably synthesized in the tapetum. The yellow staining is associated with exine development of microspores (Ahokas 1976). Apparently the developmental disorganization of the tapetum results in disturbances, in de novo synthesis of esterases. Low activity of esterases at later stages in the anther development is reported in CMS wheat (Hohler and Borner, 1980) and CMS *Petunia* (Van Marrewijk et al., 1986). In mutant tomato (Sawhney and Bhadula 1988), delay in the tapetal degeneration and absence of exine deposition are associated with low esterase activity. Therefore enzyme esterase is localized in the tapetum before its degeneration; at the time of formation of microspores release (Vithanage and Knox 1976, 1979; Sawhney and Nave 1986). It is possible that esterases are involved in the polymerization of sporopollenin and deposition of primexine. It has been showed that esterase activity was lower in CMS anthers than that of the normal ones (Bhadula and Sawhney 1987). It appears that the abnormal development of tapetum is correlated with low activity of esterase. In part, such state is responsible for the abortion of many microspores in mutants.

In *Pennisetum* fertile anthers, esterase activity is high in the tapetum at microspore stage, and is possibly associated with exine formation. But, in male sterile anthers, esterase activity in tapetal cells is negligible and sometimes not visible at all. Delay in tapetal degeneration and absence of exine deposition due to disturbance in esterase activity is, therefore, closely associated with sterility.
Biochemical:

Plants have their own independent genetic and protein synthesizing mechanism in the nucleus as well as the cytoplasm. Cytoplasmic organelles, particularly mitochondria and chloroplasts, are the major active conversion sites with vital role because of the presence of specifically unique DNA, RNA, ribosomes and enzymes in them. Chloroplast DNA (cpDNA) of higher plants is considered to be highly conservative. This is because it exists in multiple copies per organelle (Herrmann 1970; Kirk 1971). On the other hand, mitochondrial DNA (mtDNA) genome of higher plants is conspicuously large and exhibits considerable size variation and genetic diversity. Even in closely related species, wide variations in complexity is evident. Large number of studies have suggested that ge-mst in maize and Sorghum is due to mutations in mt-DNA. The main evidence is based on the mt-DNA structure, its restriction endonuclease fragment analysis, translation production, diseases susceptibility and chemical effects.

On the basis of bio-chemical studies, mitochondria have been strongly implicated for cytoplasmic male sterility. Mitochondria have their own limited nucleic acid system synthesising 10-12 polypeptides (Leaver and Forde 1980). The inner membrane of mitochondria is made up of enzyme complexes and is involved in oxidative phosphorylation and generation of ATP (Leaver and Forde 1980). Aberrant synthesis of any one of the mitochondrial encoded proteins can initiate the developmental abnormalities either in the tapetum or in sporogenous meiocyte cells resulting in male sterility. Restriction endonuclease fragment analysis of mitochondrial DNA has shown that mitochondrial genome and its products are different from those of fertile anthers as in CMS-I, CMS-C and CMS-S maize (Leaving and Pring, 1976; Pring and Leavings, 1978; Laughnan and Gabay-Laughnan 1983).

Specific differences in cytochrome oxidase content and isoenzymatic bands occur between mst and mft anthers of maize, rice, Sorghum and wheat, but no such differences occur in succinate dehydrogenase, because cytochrome oxidase is
partially mt-coded and succinate dehydrogenase is a nuclear genome coded enzyme. Thus, a significant difference in cytochrome oxidase during development of mt-DNA is seen in mst Sorghum (Alam and Sandal 1969), rice (Anonymous 1977) and maize (Ohmasa et al., 1976) anthers. Abnormal cytochrome oxidase system lead to defective oxidative phosphorylation. Comparing mst and mft wheat anthers, Shrivastava (1981) found significant differences not only in cytochrome oxidase but also in other biochemical functions of mitochondria like oxidative phosphorylation and ATPase activity. Hence, constant and specific biochemical differences exist between the mitochondria of fertile and those of sterile anthers.

Characterization of anther proteins by polyacrylamide gel electrophoresis reflects the differences in the protein profile of fertile and CMS anthers. Markova and Daskaloff (1976) reported 15 and 17 protein bands, respectively in CMS and normal lines of Capsicum. Similarly, in Sorghum, Alam and Sandal (1969) reported 20 bands in fertile and 9 bands in sterile line. In petunia (Wu and Murray 1985), absence of 63 and 45KD proteins is reported during meiosis where pollen abortion begins in sterile line. In tomato (Bhacdula and Sawhney 1991), the mutant stamens contain low levels of soluble proteins which are related to reduction in protein synthesis. The mutant stamens, however, possessed many poly-peptides similar to the normal, and synthesized a 53 KD polypeptide at stages when there are abnormalities in tapetal development. The mutant stamens also possessed a 23 KD and some low molecular weight polypeptides that were considered as degradation proteins. Normal stamens exhibited the synthesis of many poly-peptides which are not found in the mutant, from microspore mother cell to the pre-anthesis stages. In addition, at the time of pollen maturation, there was a greater synthesis of several polypeptides, particularly 42 and 37 KD. Although the causative mechanisms of male sterility in the S/-2S/-2 mutant are not known, the synthesis and lack of specific polypeptides, reported here, appear to be associated with pollen degeneration. In barley, Ahokas (1980) observed specific protein differences during post-meiotic stages of anther development where abnormal deposition of sporopollenin is
reported in CMS line. In the present study, in both *Raphanus* and *Pennisetum*, differences in protein profiles are observed between fertile and sterile anthers. However, it is difficult to attribute these differences to pollen abortion as the fertile anther at anthesis synthesizes many pollen specific (gametophytic) proteins. As a result, fertile anthers contain more protein bands than the sterile ones where microspores are degenerated.