CHAPTER-II

REVIEW OF LITERATURE
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Anatomical, cytological and biochemical aspects of male sterility have been reviewed extensively by Edwardson (1956, 1970), Laser and Lersten (1972), Frankel and Galun (1977), Kaul (1988) and Hegde and Isaacs (1992). These studies have revealed varied types of mechanism of male sterility in different plants, and sometimes in the same species, and occasionally even in the same flower.

According to the hypothesis proposed by Frankel (1971) the formation of a functional and non-functional pollen grains is determined by the presence of “fertility” and “sterility” elements, respectively in the cytoplasm of cells. The “fertility elements” are present in an integrated state while the ‘sterility elements’ are in an autonomous state. Both these elements may occur in the cells of same plant (Evenor and Izhar, 1984). The fusion between the cells containing ‘fertile’ and ‘sterile’ elements results in the formation of cybrids containing both the types of elements (Izhar and Tabib, 1980). Such heteroplasmic cybrids are fertile (Izhar et al., 1983). The heteroplasmic cybrids segregate into homoplasmic male sterile plants in subsequent generations (Izhar et al., 1983). The site of the sterility elements is presumed to be mitochondria and occasionally chloroplasts (Izhar, 1984).

The association between cytoplasmically inherited mutant organelles and male sterility is envisaged in many plants. According to Flavell (1974), in maize, the presence
of a hypothetical 'anther specific' substance makes mutant organelles functionless. But these anther specific substances have no effect on the activities of the normal organelles present in the fertile anthers. The organelles can be reverted back to functional state by altering the binding site of the anther specific substances through nuclear restoration genes. The nuclear restoration genes are also presumed to be involved in the production and transfer of the anther specific substances (Flavell, 1974).

According to Levings (1993) the cause for male sterility in CMS-T maize lies in its sensitivity to a pathotoxin. URF-13 mitochondrial gene, in this sterile mutant, is known to interact with toxin to cause pores in membrane. According to Levings (1993) the anther specific substance (Flavell, 1974) is expected to interact with URF-13 protein to permeabilize the inner mitochondrial membrane resulting in mitochondrial dysfunction and cell death, particularly of tapetal cell layer. The anther-specific substance seems to have properties similar to T-toxins or methomyl whose expression is limited to the anther whereas URF-13 gene is present in other plant organs also. Because anthers are known to carry out specialized functions and that unique components are synthesized in them, such as sporopollenin, it is feasible to presume the existence of anther specific substance. According to Levings (1993) this model is attractive because URF-13 is known to destroy mitochondrial activity and cause cell death which subsequently lead to pollen abortion. But, attempts are not successful to isolate anther specific compound with properties of T-toxins or methomyl. However, studies on male sterile Vicia faba (Edwardson et al., 1976;
Scalla *et al.*, 1981; Moussel *et al.*, 1982) have provided evidence for the presence of sterility associated specific substances. These workers observed what are known as cytoplasmic spherical bodies (CSBs) in the tissues of CMS *Vicia faba*. CSBs are seed-transmitted bodies consisting of central fibrous core surrounded by a double membrane. They are 62 nm in diameter and made up of a circular unit membrane enclosing an electron dense zone. CSBs contain a single stranded RNA (Scalla *et al.*, 1981). Sequential progress in growth of the sterile anther correlate with increase in the number of CSBs reaching maximum in the microspores (Moussel *et al.*, 1982). Since they are observed only in the cells of male sterile *Vicia faba*, and not in the cells of fertile line, CSBs are considered as the sites or products of sterility factors (Edwardson *et al.*, 1976). According to Scalla *et al.* (1981) CSBs function like viruses and carry the genetic determination of sterility.

I. Developmental and anatomical aspects of male sterile anthers

Anatomical studies on male sterile anthers have helped to understand the structural aberrations of anther tissues at cell level and the developmental stage at which they occur. These studies also help in obtaining the information on vital aspects of successful reproduction in normal plants.

The breakdown of microsporogenesis may occur at any stage of anther development. In rice, it occurs either at tetrad stage or at microspore stage or at pollen
stage (Pradhan, 1992). Some mutants of barley show suppression of meiosis and subsequent degeneration of meiocytes (Kaul and Sudha, 1990, 1991). Degeneration of microspores, prior to or immediately after their release from tetrads is also reported in mutants of barley (Singh and Kaul, 1990; Kaul and Singh, 1991). Meiotic irregularities such as univalents, irregular segregation of chromosomes at meiosis I and II, micronucleus, chromosome breaks and bridges, sticky chromosomes, absent or defective cytokinesis, cell fusion, chromatin degeneration and pycnosis, abnormal spindles, giant cells are reported in *Hevea brasiliensis* (Saraswathy Amma et al., 1990), partial male sterile maize (Defani-Scoarize, 1995), *Centella asiatica* (Consolaro and Pagliarini, 1996) and *Lathyrus odoratus* (Seijo, 1996). In *Brassica napus* (Junica, Napus, Polyma and Resyn genotypes) of sporogenous cells fail to differentiate in the anther (Theis and Robbelen, 1990). Stamen primordia fail to differentiate into anther and filament, and fuse with ovary wall producing an abnormal tissue in CMS *Nicotiana tabacum* (Hegde et al., 1992; Hegde et al., 1996).

In many male sterile anthers tapetum becomes abnormal, structurally and functionally. The nature and stage at which abnormality is manifested varies from species to species. In CMS *Capsicum annuum* (Horner and Rogers, 1974), different CMS lines of *Helianthus annuus* (Horner, 1977; Hegde and Isaacs, 1992; Kini et al., 1994), CMS rye (Scoles and Evans, 1979), *Impatiens* (Van Went, 1981), rice (Nishiyama, 1984), stamenless-2 mutant of tomato (Sawhney and Bhadula, 1988) and *Bidens* (Sun and
Ganders, 1987) tapetal cells enlarge radially and become vacuolated at tetrad stage. The hypertrophied tapetum obliterates the development of microspores in more than one way. The enlargement of tapetal cells results in the reduction of space in the anther cavity. As a result the primexine-bound microspores experience physical constraint of morphogenetic significance. They collapse within the callose wall. In CMS HA-232 sunflower (Horner, 1977) the hypertrophied tapetum lacks cell walls and organells. In CMS 234 sunflower (Hegde and Isaacs, 1992) the hypertrophied tapetum contains an intact cellulosic wall. Tapetum with dense inner and radial walls is also reported in CMS-C Zea mays (Lee et al., 1979). According to Chauhan (1977) persistent abnormal tapeta fail to provide nutrition to the developing microspores. The failure to develop endotheccial thickenings in many sterile anthers is attributed to the effect of inhibitory substances synthesized by the persistent tapetum (Chauhan, 1977; Scolas and Evans, 1979; Dundas et al., 1981).

In a few male sterile anthers structural abnormality is manifested by the persistence of middle layer. In male sterile sunflower (Nakashima and Hosokawa, 1974a; Hegde and Isaacs, 1992), subsequent to the degeneration of the hypertrophied tapetum, the cells of the middle layer enlarge and invade the anther locule. In Cajanus cajan (Dundas et al., 1981) the tapetum degenerates prematurely and out of two middle layers the inner one becomes hypertrophied. The outer middle layer persists beyond its schedule. In rye (Scoles and Evans, 1979), subsequent to degeneration of tapetum and microspores into an unorganized mass, the middle layer breaks down.
Abnormality in the tapetal cells of male sterile anthers is expressed at higher level of organization also. In CMS Zea mays, tapetal cells show vacuolation and cytoplasmic disintegration prior to their breakdown along with middle layer (Cheng et al., 1979). In CMS-T line of Zea mays, tapetal mitochondria lose their internal structure, increase in size and become sac-like (Warmke and Lee, 1977). In CMS-C line exine development is either delayed or inhibited due to irregular deposition of Ubish bodies by the tapetum. In mutants of barley tapetum becomes thin-walled (Singh and Kaul, 1990, 1991; Kaul and Singh, 1991). In male sterile Brassica napus genotype Takagi (Theis and Robbelen, 1990) tapetum possesses toluidine blue-positive outer surface layer which resembles sporopollenin precursors. In Houttuynia cordata (Takashashi, 1986) tapetal cells do not show any demonstrable abnormalities. In ms2 mutant of Glycine max (Graybosch and Palmer, 1985a) the tapetal abnormalities are expressed by premature vacuolation, persistent inner tangential wall, failure to differentiate normal quantity of endoplasmic reticulum and dictyosomes, disruption of plastids and premature degeneration of cells. In ms3 mutant of Glycine max (Graybosch and Palmer, 1987) tapetal cells either collapse or possess electron dense material which stains and fluoresces similar to sporopollenin. According to Graybosch and Palmer (1987) this suggests the possibility of blocking of intercellular transport of sporopollenin precursors from the tapetum of microspores. In a new GMS line of Glycine max, tapetal cells show unusual formation of vacuoles, disruption of organelles and accumulation of densely staining material (Wei, Palmer and Horner, 1996; Wei et
In male sterile *Cajanus cajan* (Dundas et al., 1981) tapetum undergoes a precocious degeneration. But in another GMS line of the same species Katti et al. (1994) report persistence of tapetum until final maturity of the sterile anther. In CMS *Beta vulgaris* (Hallden et al., 1991) walls and membrane of tapetal cells disorganize and dissolve. Tapetal cytoplasm in this plant shows no distinct degeneration even after microspore formation. In CMS *Petunia hybrida* (Van Went et al., 1986), at leptotene stage, tapetal cells become smaller with large vacuoles and elongated mitochondria which contain serial tubular cristae. At anaphase-I, tapetal cells become deformed with disrupted nucleus and disorganized cytoplasm. In ms-25 and ms-26 *Zea mays* microspores abort soon after release from the tetrad (Loukides et al., 1995). ms-25 mutant shows large lipid bodies in the tapetum at vacuolate stage of dying microspores and ms-26 shows large vacuoles in both tapetal cells and young microspores. According to Loukides et al. (1995), because both mutants show abnormal tapetal cell morphology, mutations affect the expression of genes in tapetal cells.

The breakdown of microsporogenesis in male sterile plants also results due to other factors. Among them is the mis-timing of callose deposition and/or its dissolution. Precocious dissolution of callose is reported in male sterile *Petunia hybrida* (Izhar and Frankel, 1971), transgenic *Nicotiana tabacum* (Worrall et al., 1992) and *Oryza sativa* (Agadi, 1996). Persistence of callosic wall around tetrads is reported in *Petunia hybrida* (Van Went et al., 1986), *Helianthus annuus* (Horner, 1977; Hegde and Isaacs, 1992),
ms₂ mutants of Glycine max (Grýbosch and Palmer, 1985a, 1987), a new GMS mutant of Glycine max (Wei, Palmer and Horner, 1996), Brassica napus genotype Takagi (Theis and Robbelen, 1990) and Cajanus cajan (Katti et al., 1994). In Impatiens (Van Went, 1981) male sterile anther shows slow dissolution of callose. The mistiming of callose dissolution is considered as a result of malfunctioning of tapetum. It appears that the timing of callase secretion by tapetal cells is very critical for normal pollen development. How the mistiming of callase activity is brought about in male sterile plants is not clear. In normal Petunia, the pH of anther locule drops from about 7 to 6 towards the end of the tetrad stage, just before callase becomes active (Izhar and Frankel, 1971). In CMS Petunia, in which callase is activated prematurely, the pH drop occurs early in meiosis (Izhar and Frankel, 1971). According to Wei et al. (1996), in Glycine max, callase gene is present in both normal and sterile lines. Reduction in callase mRNA in the sterile line probably suggests abnormal transcription.

The contention that premature callose dissolution leads to collapse of the developing microspores (Izhar and Frankel, 1971) has been confirmed in transgeneic tobacco plants (Worrall et al., 1992). When a modified callase gene, known to be active in the tapetum during meiosis, is fused to an Arabidopsis promoter, a partial or complete male sterility is observed in transgenic tobacco plants. Meiosis is apparently normal in the absence of callose wall, but not the pollen wall development. This study has shown that the absence of callose causes abnormality in the microspores and tapetal abnormality is simply a
consequence of a disruption in microspore development. This means that the tapetal cells do not function independent of the microspores. In other words, the two cell types are somehow interdependent during pollen development.

The apparent cause for pollen sterility in ms1 mutant of *Glycine max* (Albertsen and Palmer, 1979) is aberrant or incomplete cytokinesis. Such abnormality is also reported in ms4 mutant of *Glycine max* (Graybosch and Palmer, 1985b), *Houttuynia cordata* (Takahashi, 1986) and bean (Johns et al., 1992). The coenocytic microspores in *Glycine max* (Albertsen and Palmer, 1979) possess pollen grain type wall and food reserves. They also show persistent cytoplasmic channels composed of continuous layers of ektexine and endexine from each coenocyte microspore. In *Houttuynia cordata* (Takahashi, 1986) coenocytic microspores show wide range of variations in shape and size and contain only ektexine. They degenerate at vacuolate stage. In ms4 mutant of *Glycine max* microspore exine lacks sporopollenin (Graybosch and Palmer, 1985a). The development of abnormal pollen wall appears to be a common feature in many male sterile plants. For example, in ms2 *Glycine max* (Graybosch and Palmer, 1985b) microspores degenerate immediately after formation of primexine and probaculae. The microspores of stamenless-2 mutant of tomato (Sawhney and Bhadula, 1988) and *Oenothera* (Noher de Hallac et al., 1990) possess no exine at all. These naked microspores become target for hydrolytic enzymes present in the thecal fluid. In *Vicia faba* (Audran and Willemse, 1982) the sterile pollen...
grains show absence of exine materials. Exceptionally, the pollen grains of CMS *Raphanus* (ms NPZ) possess well formed exine (Theis and Robblen, 1990).

Variations in the breakdown stages of microsporogenesis may occur in the same species and sometimes even in the same flower. In ethyl methane sulphonate, diethyl sulphonate and gamma-rays induced sterile anthers of *Pisum sativum* (Nirmala and Kaul, 1991) disruption of microsporogenesis occurs at pre- or post-meiotic periods. In CMS *Nicotiana tabacum* cultivar NPN-190 (Hegde et al., 1996) variations in the stamen morphogenesis are observed in a single flower. Some stamen primordia fuse with gynoecium while others develop into branched or unbranched structures - one branch developing into a stigmatoid stamen and another into a carpelloid stamen. The carpelloid stamen bears naked ovules on them.

Variation in the stage of breakdown of microsporogenesis in five different plasma types of *Petunia hybrida* is presumably due to genetic variation in them (Izhar and Frankel, 1976). In all the five plasma types, the first signs of breakdown of microsporogenesis are at early prophase in the form of changes in free amino acids composition. It is postulated that a single plasmagene induces male sterility in *Petunia*. According to Izhar (1977), variation in the breakdown time of the microsporogenesis is due to interaction of this single plasmagene with genes that control the breakdown time. These genes operate as
temperature sensitive genes. Different amounts of alleles interact at different temperature, leading to wide variations in the phenotypic expression of male sterility (Izhar, 1977).

Variations in the timing of breakdown of microsporogenesis is also observed in different cytoplasmic genotypes of Zea mays. In CMS-T Zea mays (Warmke and Lee, 1977) tapetum abnormality occurs at tetrad stage. In CMS-C type of Zea mays (Lee et al., 1979) the tapetum manifests abnormality either at intermediate microspore stage or at early tetrad stage. In the same sterile line Colhoun and Steer (1981) report abortion either at meiocyte or at dyad/tetrad or at microspore stage in different anthers. According to Colhoun and Steer (1981) mitochondrial degeneration in the tapetum is not necessarily the earliest detectable event in the process of pollen sterility. A reduction in, or a complete failure of, formation of orbicules is also observed in CMS-T anthers.

In the opinion of Colhoun and Steer (1981) the possible mechanisms of sterility in CMS Zea mays are not restricted to only two types as proposed by Lee et al. (1979). In contrast to the earlier reports (Lee et al., 1980), Colhoun and Steer (1981) claim that in CMS-S Zea mays the pollen abortion occurs at vacuolate microspore stage. But according to Lee et al. (1980), in CMS-S Zea mays pollen abortion occurs at very late stage of anther development. Unlike C- and T-types of mutants, tapetum in CMS-S Zea mays appear normal. Therefore, Lee et al. (1980) presume that male sterility in CMS-S plants is determined by the pollen itself without external influence from the tapetum.
Variation in the breakdown stages of microsporogenesis in different locules of the same anther has been reported in CMS V-20 rice (Agadi, 1996). This sterile line shows degeneration of callose-less meiocytes in one locule; degeneration of young microspores in another; and degeneration of old vacuolate microspores in some other locule. According to Agadi (1996) a close correlation exists between tapetal abnormality and stage of breakdown of microsporogenesis.

II. Cytochemical (histochemical) studies on male sterile anthers

Often the aberrations in the male sterile anthers are expressed in the form of chemical alterations. These alterations reflect the possible role(s) of chemical substances in the growth and maturation of normal and male sterile anthers. In most of the cases the chemical alterations appear as consequences of male sterility rather than causes.

The nutritional imbalance in male sterile anthers stem from several origins. In CMS Beta vulgaris (Rohrbach, 1965), Triticum (Joppa et al., 1966) Sorghum (Alam and Sandal, 1967) and Oryza (Agadi, 1996) poorly developed vasculature in the stamens is considered as a cause for nutritional imbalance. Poor development of vasculature is believed to impede or block the transport of nutrients thereby resulting in inadequate accumulation of nutrients in the anther tissues.
There are few autoradiographic studies which support the theory of nutritional blockade in male sterile anthers. In CMS sugar beet, in comparison with male fertile lines, relatively more accumulation of $^{14}$C compounds is observed in the tapetum at tetrad stage (Nakashima and Hosokawa, 1971). The ground tissue of the connective of sterile anthers possess much less concentration of $^{14}$C compounds at microspore stage. According to Nakashima and Hosokawa (1971), in the sterile anthers, nutrients are not transferred from the abnormal tapetum to microspores. This leads to the starvation of microspores and consequently their abortion. Low accumulation of $^{14}$C compounds is also reported in the male sterile anthers of maize (Criswell et al., 1974). In two GMS lines of rye (Cebrat and Zadecka, 1978) abnormal vasculature of the connective appears to be responsible for blockade of nutrients. In these lines, tissues surrounding the vascular bundle of the anther either develop into endoderm-like cells or their cell walls become suberized. However, according to Cheng et al. (1979), the defective physiological condition in the anther may also result from hormonal imbalance.

Fertile and sterile anthers showing quantitative difference in the accumulation of nutrients has been reported for several other species also. In Sorghum and Zea mays the developing male sterile and fertile anthers show a gradual increase in the reducing sugars. But the storage starch persists in the endothecium and connective in fertile line, and disappears in the CMS line (Nakashima and Hosokawa, 1970). In male sterile Pennisetum persistence of starch is observed in the endothecium at dyad stage (Khattra and
Singh, 1989). Hegde and Isaacs (1992) have also observed persistence of starch storage at tetra$ stage in the CMS sunflower anthers. It is inferred by these authors that the persistent callose around the tetrads functions as a barrier preventing the transport of nutrients into the anther locule. Subsequent disappearance of starch storage from sterile anthers is correlated with the hypertropy of the tapetum and middle layer. Similar report is also available in cool-induced male sterile rice anthers which show an increase in non-reducing sugars and starch, decrease in inorganic phosphate and acid phosphatase activity, and degeneration of microspores (Nishiyama, 1984). The tapetum becomes dilated showing condensed cytoplasm accompanied by augmentation of motochondria, proplastides, Golgi bodies, vacuoles and endoplasmic reticulum. According to Nishiyama (1984) these indicate an inhibition of transportation of nutrient substances from tapetum to microspores. This results in the poor growth of the microspores and dilation of the tapetal cells.

Many male sterile anthers exhibit low activity of enzymes. For example, activities of succinic dehydrogenase (Fukasawa, 1961) and acid phosphatase (Chauhan and Singh, 1968) are low in the anthers of male sterile Cucumis melo. Male sterile anthers of maize contain low amylase activity and high polyphenol oxidase and peroxidase activities (Dmitrieva and Khavzhinskayia, 1962). Male sterile stamenless-2 mutant of tomato (Sawhney and Bhadula, 1987) shows low esterase activity which correlates with delayed degeneration of tapetum and failure of exine deposition. These abnormalities are
considered responsible for pollen degeneration. In CMS sunflower, prior to visible structural deviations, sporogenous cells show low activities of succinic dehydrogenase, cytochrome oxidase and malate dehydrogenase (Hegde and Isaacs, 1992). The effect of altered callase activity on pollen development is already mentioned.

A comparative account of free amino acid composition in male sterile and fertile lines is available for several plants. In male sterile wheat (Fukasawa, 1954) and apple (Tupy, 1963) proline content is low whereas in Zea mays and Sorghum (Nakashima and Hosokawa, 1970; Nakashima, 1975) and Raphanus (Ogura, 1968) this amino acid is totally absent. Male sterile lines show more aspartic acid and alanine in Beta vulgaris (Rohrbach, 1965) and glycine in Sorghum vulgare (Brooks, 1962). On the other hand male sterile lines of Sorghum vulgare var. sudanese contain low quantities of alanine, glutamic acid, proline, phenylalanine and tyrosine (Alam and Sandal, 1972). Male sterile lines of Sorghum also contain low histidine, threonine, glutamic acid, leucine and phenylalanine, but higher concentration of alanine, serine, proline and tyrosine (Tripathi et al., 1981). According to Tripathi et al. (1981) the difference in the amino acid composition in the anthers of sterile and fertile lines are suggestive of their involvement in growth and development. According to Izhar and Frankel (1973) amino acid imbalance probably induces changes in pH in the anthers of fertile and sterile lines.
In the anthers of CMS barley abnormality is expressed by excess and uncontrolled secretion of sporopollenin (Ahokas, 1978). It seems that energy of the CMS anthers is spent mainly in the process of biosynthesis of sporopollenin precursors. Consequently several other abnormal features appear such as poorly developed or absence of endothecial thickenings and terminally swollen endothecial chloroplasts. Chloroplasts often degenerate or transform into yellow or pink chromoplasts. The cells of the middle layer collapse early and show degeneration of cell organelles. The exine of the sterile microspores grows rapidly and becomes 2-5 times thicker than that of normal pollen grains. The anther lobes collapse and exine of microspores fuse with the locular sporopollenin and locular wall.

Absence of endothecial fibrous walls and reduced cuticle production on epidermis are reported in the anthers of GMS Zea mays (Cheng et al., 1979). Starch accumulation is observed in the epidermis and endothecium of both fertile and sterile anthers at the pre-callose stage. This accumulation persists for 6-8 days and subsequently disappears. Starch increases and declines prior to and during meiosis, respectively. Starch is considered as a prerequisite for later synthetic process such as endothecial wall formation, epidermal cuticle elaboration, pollen wall formation and pollen starch accumulation. According to Cheng et al. (1979) starch accumulated in the endothecium is not exclusively used for the synthetic activities in these cells. Because, in fertile anthers of Zea mays, although starch deposition is present in all the endothecial cells only at the anther tips fibrous thickenings
are developed. In the sterile corn anthers no endothecial thickenings differentiate, but nevertheless the cells accumulate starch grains (Cheng et al., 1979).

In Triticum, Hordeum, Avena, Zea, Helianthus, Petunia and Trifolium normal development of anther is hampered by the copper deficiency and the degree of male sterility is directly proportional to the degree of deficiency of copper (Dell, 1981). With increase of copper supply a progression is observed from number of flower formation, to-staminodes only, to anthers without tetrads, to anthers with sterile pollen grains and reduced lignification, to normal anthers in copper-adequate plants. The activity of some copper containing enzymes such as catechol oxidase appears to be low in copper-deficient plants. Catechol oxidase is associated with biosynthesis of lignin. According to Dell (1981) the degeneration of pollen grains occurs due to lack of mobilisation of copper at critical stage of microsporogenesis.

CMS Petunia anthers differ from fertile and restorer anthers in having relatively low esterase activity (Van Marrewijk et al., 1986). In male fertile anthers esterase activity, during late prophase to early microspore stage, is concentrated in the outer tapetal layer. In male sterile anthers tapetal breakdown correlates with non-accumulation of esterases. The difference in cytochrome oxidase activity occurs from meiosis onwards and only after the appearance of initial symptoms of degeneration (Bino, et al., 1986). According to Van
Marrewijk et al. (1986) the difference in esterases and cytochrome oxidase activity are effects rather than a cause of the pollen sterility in *Petunia hybrida*.

However, the above speculation does not hold good for male sterile maize. Anthers in this plant show reduced activity of cytochrome oxidase from pre-meiosis onward (Bino, *et al.*, 1986). In addition, later, the mitochondrial localization of cytochrome oxidase differs between pollen of fertile and sterile anthers. In fertile pollen cytochrome oxidase activity is localized in the cristae and within the space between the outer and inner limiting membranes of the organelles. In sterile pollen cytochrome oxidase activity is observed only between the outer and inner membranes of mitochondria. Since these differences occur prior to visible structural signs of degeneration, Bino *et al.* (1986) suggest that male sterility in maize correlates with deviations in cytochrome oxidase activity.

Comparative analysis of the activity of amylases, and level of starch and soluble sugar content in the normal, 'gibberellin-sensitive', and GA$_3$-reverted mutant stamens of tomato (Bhadula and Sawhney, 1989) has shown that the sterile stamens after meiosis possess significantly low amylolytic activity. Starch content decreases in normal anthers at later stages while storage persists in mutant stamens. The mutant stamens also contain a steady, but low, levels of soluble sugars. In GA$_3$-reverted mutant stamens, the amylolitic activity and the level of starch and soluble sugars are comparable to normal stamens. According to Bhadula and Sawhney (1989) sterile stamens contain low levels of
endogenous gibberellins which affect the activity of amylases. Reduced activity of amylases results in lower sugar levels which ultimately leads to abnormal pollen development.

According to Kaul and Sudha (1990) a reduced biochemical components are regular features of male sterile anthers. Mutant anthers of barley contain relatively less carbohydrate and protein metabolism. Since these biochemical components are essential to provide nutrition and energy, their depletion results in the starvation of the developing microspores leading to their death. According to Markova (1990) the disturbances in carbohydrate metabolism in the CMS anthers may not be due to deficiency in monosaccharides. The disturbed carbohydrate metabolism may result from the normal phosphorylation of hexoses which is due to lower levels of hexokinase activity. However, whether such alterations in the carbohydrates and proteins are the cause or consequences of male sterile gene action is not determined.

Estimation of free putrescine, spermidine and spermine levels and the activities of ornithine decarboxylase and $s$-adenosylmethionine decarboxylase, at intermediate temperature, in the floral organs of the normal and male sterile stamenless-2 mutant of tomato (Rastogi and Sawhney, 1990a) has shown that all mutant flower organs contain significantly higher levels of polyamines and enzyme activity than their normal counterparts. However, at low temperature, the reverted mutant stamens show polyamine levels and
activity of polyamine biosynthetic enzymes on par with normal stamens. Therefore, it is suggested that the abnormal stamen development in the male sterile mutant of tomato is, in part, related to high levels of endogenous polyamines. Further, Rastogi and Sawhney (1990b) have shown that the polyamines act as inhibitory agents for in vitro growth and development of both normal and mutant flower buds. The inhibitors of polyamine biosynthesis induce the formation of normal-looking pollen in the stamens of mutant flower buds. Since polyamine induces abnormal stamen development in the normal flower, and inhibitors induce normal-looking pollen in mutant flower buds, Rastogi and Sawhney (1990b) suggest that the high levels of polyamines are responsible for abnormal stamen development.

In vitro growth and development of flower buds of male sterile stamenless-2 mutant of tomato are dependent on the presence of benzylaminopurine and gibberellic acid (Sawhney and Rastogi, 1990). But, for in vitro growth of normal flower buds only benzylaminopurine is required. The mutant buds grow in 4-5% sucrose but not the normal buds. Based on these observations, Sawhney and Rastogi (1990) suggest that the mutant flower buds contain low levels of endogenous gibberellins. Since gibberellins induce the synthesis of amylases, which in turn are responsible for the breakdown of starch to provide free sugar for the developing organs, the low levels of gibberellins present in the mutant bud produce inadequate quantity of soluble sugars affecting the normal development of pollen grains.
Cytochemical analysis of microsporogenesis of wild type and nuclear male-sterile mutant of *Arabidopsis thaliana* (Regan and Moffatt, 1990) has shown lack of adenine phosphoribosyl transferase (APRT) in the mutant. Alterations in the microsporogenesis occur in the mutant line just after the dissolution of callose around microspore tetrads. Due to incomplete synthesis of intine, microspore wall stains dark. Microspores show irregular and delayed vacuole formation and absence of mitotic division. After meiosis, in addition to reduction in RNA accumulation, the mutant anthers show reduced activity of alcohol dehydrogenase and esterases. During development of the normal anther free adenine, produced from the breakdown of purine nucleotides and nucleotide factors, is rapidly salvaged by APRT. In the mutant stamens adenine is not salvaged because they lack APRT. Due to the presence of callose and/or exine, adenine and one of its degradation products accumulate to toxic levels in the mutant anther tissues. Therefore, Regan and Moffatt (1990) suggest that abnormal pollen development in the APRT deficient mutant is due to the poisoning of the anther tissue due to the deficiency of nucleotide. In addition, the deficiency of APRT activity in the mutant may also alter the metabolism of cytokinin bases and thus the profile of cytokinin metabolites.

In mutant sterile anthers of *Oenothera*, chemical alterations in the callose are associated with male sterility (Noher de Halac et al., 1990). Callose at the middle tetrad
stage stains weak. At this stage the tapetal cells contract and develop dark inclusions and pycnotic nuclei.

Report from Singh et al. (1992) reveals that stamens of normal and male sterile stamenless-2 mutant of tomato contain different levels of endogenous IAA. At low (18°C/15°C; day/night), intermediate (23°C/18°C; day/night) and high (28°C/23°C; day/night) temperature the mutant leaves show 10-20 times higher IAA concentrations than the normal leaves. At intermediate and high temperatures, the mutant stamens contain 5-8 times higher IAA concentrations. At low temperature reverted mutant stamens show IAA level similar to that in normal stamens. Therefore, it is proposed that the environmental factors induce changes in the levels of endogenous plant hormones and higher IAA content in leaves and stamens of the mutant is one of the factors associated with male sterility and carpelization of stamens (Singh et al., 1992).

Differences between male sterile and fertile lines, in the levels of other growth regulators are also reported. In CMS Brassica napus (Shukla and Sawhney, 1992) the leaves contain highest levels of cytokinins as compared to the other organs. The normal line shows higher levels of cytokinins in the root, stem and mature flowers. These authors implicate that the lower levels of cytokinins in the flowers of CMS line of Brassica napus are likely involved in the expression of male sterility.
III. Biochemical studies on male sterile anthers.

Biochemical studies help to understand the molecular events that restrict the phenotypic consequences of nuclear-cytoplasmic defects to specific anther developmental stages. These studies also help in the identification of gene products involved in pollen development. Biochemical studies have shown that abnormal development of male reproductive tissues is correlated with modifications of the mitochondrial genome (Boutry and Briquet, 1982; Hanson and Conde, 1985). An association between unusual mitochondrial proteins and CMS phenotype has been identified in *Vicia faba* (Boutry and Briquet, 1982), different lines of *Zea mays* (Laughnan and Gabay-Laughnan, 1982; Dewley et al., 1987; Wise et al., 1987; Hack et al., 1991), *Petunia hybrida* (Izhar et al., 1983; Evenor and Izhar, 1984), *Lycopersicon esculentum* (Bhadula and Sawhney, 1991), *Helianthus* (Horn et al., 1991; Laver et al., 1991; Moneger et al., 1994; Smart et al., 1994), *Capsicum* (Manoharan et al., 1993) and bean (Johns et al., 1992; Abad et al., 1995). All these studies strongly support the mitochondrial location of genetic determination of CMS phenotype. In CMS lines of *Sorghum bicolor*, Sane et al. (1994) implicate relationship between polymorphism in mitochondrial genes and ATP synthase complex. They propose that mitochondrial gene products are incompatible with the complimentary products produced by the nuclear genome. Compatibility depends upon presence of complimentary mitochondrial gene products to make ATPase enzyme. The defect assembly, even if functional, would be inefficient and hence could lead to male sterility (Sane et al., 1994).
In male sterile oilseed rape, the fertility is restored by a chimaeric ribonulease-inhibitor gene (Mariani et al., 1992). A cross between male sterile plant expressing a chimaeric ribonuclease genes in the anther tapetal cells and male fertile plant that was transformed with a chimearic tapetal cells specific ribonulease-inhibitor gene produced fertile $F_1$ plant by the suppression of cytotoxine ribonulease RNase/RNase inhibitor complexes.

In transgenic Petunia inhibition of pigment synthesis causes male sterility (Van der Meer et al., 1992). This has led to the implication that flavonoids play an essential role in male gametophyte development, in addition to their role in the pigmentation of flowers and fruits (Van der Meer et al., 1992).

Analysis of DNA sequences of alleles from stable fertile and male sterile progeny of transposon tagged male sterile mutant of Arabidopsis thaliana has shown that insertion of Enhancer transposable mediated inhibitor element containing gene is responsible for the male sterile phenotype. (Aarts et al., 1993).

The mitochondrial DNA in male sterile chicory plants, generated by the fusion of chicory mesophyll protoplasts and male sterile sunflower hypocotyle protoplasts, consists of a large part of the mitochondrial DNA of sunflower (Rambaud et al., 1993). The
rearrangements of mitochondrial DNA between sunflower and chicory affect the sequence of the chicory mitochondrial genes resulting in the abnormalities and sterility of chicory flowers. The intensity of the rearrangement correlates with the degree of sterility in different plants. However, it is not determined whether the appearance of sterility in chicory is due to transfer of the sunflower gene (Kohler et al., 1991; Laver et al., 1991) or whether the fusion process has given rise to a new chimaeric gene which would induce a new type of male sterility specific to chicory (Rambaud et al., 1993).

Sterility associated mitochondrial gene products are expressed in all the plant tissues. But evidences indicate that these sterility associated mitochondrial gene products affect only the formation of pollen grains whereas they are ineffective on vegetative development and female sterility (Hanson, 1991). It is presumed that this developmental specificity may be partly due to the fact that mutations in the CMS associated mitochondrial genome apparently do not disrupt other essential mitochondrial genes (Smart et al., 1994). If other essential mitochondrial genes are disrupted, the development of the whole plant would have been severely affected, as in nucleo-cytoplasmic sterile mutants (Newton et al., 1990; Rousell et al., 1991; Hunt and Newton, 1991). In CMS Vicia faba (Boutry and Briquet, 1982) a decrease in the respiratory state of oxygen uptake during oxidation of NADH or malate + pyruvate is considered as a reflection of a smaller capacity of the respiratory chain. Therefore, as implicated by Bino et al. (1985), Singh and Brown (1991) and Levings (1993), it is also possible that the respiration demand is higher in
anther tissue than in vegetative and female reproduction tissues and that mutant mitochondria in the anthers of CMS lines can not support their high demands.

Levings (1993) and Smart et al. (1994) provide explanation for tissue specificity of the mitochondria in CMS phenotypes. Plant mitochondrial genes encode polypeptides that are components of the electron transport system. Therefore, mutations among the mitochondrial genes result in the disturbance in electron transport system, ATP formation or the translation of mitochondrial messengers. These are inevitable functions needed for growth and development, and therefore mitochondrial gene mutations are deleterious and lethal. Mitochondrial gene mutations may have little or no effect on mitochondrial function in most plant cells, but may affect seriously anther cells. It is possibly because high level mitochondrial gene expression and biogenesis is required in the meiocyte cells of the anther to produce sufficient mitochondria to sustain each of four haploid microspore cells. The provision of mitochondria required for the development of microspores is greater than that of female meiocyte cells or mitotic cells of meristems because male meiocyte cell must divide equally and simultaneously into four cells, each of which becomes a male gametophyte and exists independently of the parent anther tissue. The mitochondrial mutation may disrupt or impair mitochondrial biogenesis in the meiocytes leading to cell abortion.