Review of Literature
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The body of literature on male sterility is very extensive (Edwardson, 1956, 1970; Laser and Lersten, 1972; Frankel and Galun, 1977; Kaul, 1988; Hegde and Isaacs, 1992; Smith et al., 2002). Stamens in male sterile mutants show inconsistency in ontogeny and cytochemistry, even in the same line and in the same flower.

According to the hypothesis proposed by Frankel (1971) the formation of functional and non-functional pollen grains is determined by "fertility" and "sterility" elements, respectively, present in the mitochondria, and occasionally in chloroplasts (Izhar, 1984; Evenor and Izhar, 1984). Cybrids, formed by the fusion between the cells containing ‘fertile’ and ‘sterile’ elements produce fertile pollen grains (Izhar and Tabib, 1980; Izhar et al., 1983). The heteroplasmic cybrids segregate into homoplasmic male sterile plants in subsequent generations (Izhar et al., 1983).

According to the Flavell’s hypothesis (1974), male sterility is affected by the hypothetical ‘anther-specific’ substances, which act only on mutant cell organelles making them functionless. By altering the binding site of the anther-specific substances through nuclear restorer genes the functionless organelles can be reverted back to functional state. The “anther-specific” substances seem to have properties similar to pathotoxins (Levings, 1993). ‘Anther specific’ compounds with properties of pathotoxins are not isolated. However, Edwardson et al. (1976), Scalla et al. (1981) and Moussel et al.
(1983) have reported occurrence of anther specific cytoplasmic spherical bodies (CSBs) in male sterile *Vicia faba*. CSBs are seed transmitted bodies consisting of central fibrous core surrounded by double membrane. They are 62nm 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 correlates with increase in the number of CSBs, reaching maximum in the microspores (Moussel *et al.*, 1983). Since they are observed only in the cells of male sterile *Vicia faba*, 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.

**Ontogeny and anatomy of male sterile anthers.**

Ontogenetical and anatomical studies on male sterile anthers have provided information on structural aberrations of anther tissues. The breakdown of microsporogenesis may occur at any stage of anther development. It may be at the time of differentiation of a stamen primordium, example CMS mutant of tobacco (Hegde *et al.*, 1992; Hegde *et al.*, 1996), or at the time of differentiation of sporogenous cells, example male sterile lines of *Brassica napus* (Theis and Robbelen, 1990), or at the time of initiation of meiosis, example male sterile mutants of barley (Kaul and Sudha, 1990, 1991), or during meiosis, examples *Hevea brasiliensis* (Saraswathi Amma *et al.*, 1990), partial male sterile maize (Defani-scoarize *et al.*, 1995), *Centella asiatica*
(Consolaro and Pagliarini, 1996) and *Lathyrus odoratus* (Seijo, 1996), or at tetrad and microspore stage, examples male sterile rice (Pradhan, 1992) and barley (Singh and Kaul, 1990; Kaul and Singh, 1991).

Generally the effect of male sterility is expressed in the tapetum. The nature of abnormality varies from species to species. In CMS *Capsicum annuum* (Horner and Rogers, 1974), 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), *stamenless2* 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 tapetal cells invade locular space, surround the primexine-bound microspores and exert mechanical pressure on them. In CMS HA-232 sunflower (Horner, 1977) the hypertrophied tapetum lacks cell walls and organelles. 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 endothecial thickenings in many sterile anthers is attributed to the effect of inhibitory substances synthesized in the persistent tapetum (Chauhan, 1977; Scoles and Evans, 1979; Dundas *et al.*, 1981).

Tapetal abnormality in male sterile anthers is expressed in other forms also. In the tapetal cells of CMS *Zea mays* vacuolation and cytoplasmic disintegration precede their breakdown (Cheng *et al.*, 1979). In CMS-T
Zea mays, tapetal mitochondria inflate and lose their internal structure (Warmke and Lee, 1977). In CMS-C Zea mays, exine development is either delayed or inhibited due to irregular deposition of Ubisch bodies by the tapetum. In mutants of barley tapetum becomes thin-walled (Singh and Kaul, 1990, 1991; Kaul and Singh, 1991). In Takagi mutant of Brassica napus (Theis and Robbelben, 1990) tapetum possesses toluidine blue-positive outer surface layer, which resembles sporopollenin. In MS₂ mutant of Glycine max (Graybosch and Palmer, 1985a) 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 are loaded with electron dense material, presumably sporopollenin, suggesting the blocking of intercellular transport of sporopollenin precursors from the tapetum to microspores. In another 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 al., 1996). In male sterile Cajanus cajan (Dundas et al., 1981) tapetum undergoes a precocious degeneration. In MS-Prabhat mutant of Cajanus cajan (Katti et al., 1994) tapetum is persistent until final maturity of the sterile anther. In GMS 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 GMS Petunia hybrida (van Went et al., 1986), at leptotene stage, tapetal cells become smaller with large vacuoles and
elongated mitochondria containing serial tubular cristae. At anaphase-I, tapetal
cells become deformed with disrupted nucleus and disorganized cytoplasm. In
Ms-25 mutant of *Zea mays* large lipid bodies accumulate in the tapetum at
vacuolate stage of dying microspores and in MS-26 mutant *Zea mays* large
vacuoles are formed in both tapetal cells and young microspores (Loukides *et al.*, 1995).

In the anthers of some male sterile plants abnormality is manifested in
the other wall layers. 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), premature degeneration
of the tapetum leads to the formation of hypertrophied inner middle layer. The
outer middle layer persists beyond its schedule. In rye (Scoles and Evans, 1979)
degeneration of tapetum and formation of unorganized mass of microspores are
followed by the break down of the middle layer.

Effect of male sterility on reproductive cells is not uncommon. In MS$_1$
mutant of *Glycine max* aberrant or incomplete cytokinesis are observed in
meiocytes (Albertsen and Palmer, 1979). Such abnormality is also reported in
MS$_4$ 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), formed by
incomplete cytokinesis, possess pollen grain type wall and food reserves.
Coenocytic microspores are interconnected by cytoplasmic channels composed
of ektexine and endexine. In *Houttuynia cordata* (Takahashi, 1986) coenocytic
microspores show wide range of variations in shape and size and contain only ektexine. The development of abnormal pollen wall appears to be a common feature in many male sterile plants. For example in MS₄ mutant of *Glycine max* microspore exine lacks sporopollenin (Graybosch and Palmer, 1985a). In MS₂ *Glycine max* (Graybosch and Palmer, 1985b) microspores degenerate immediately after formation of primexine and probaculae. The microspores of *stamenless2* 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 of 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 in CMS *Raphanus* possess well-formed exine (Theis and Robbelen, 1990).

In few cases variations in the expression of male sterility are found in the anthers of same flower or in the locules of same anther. In sterile anthers of *Pisum sativum* (Nirmala and Kaul, 1991) disruption of microsporogenesis occurs at pre- or post-meiotic periods. In CMS *Nicotiana tabacum* (Hegde et al., 1996) variations in the stamen morphogenesis are observed in a single flower where some stamen primordia fuse with gynoecium while others develop into branched or unbranched structures – one branch developing into stigmatoid stamen and another into a carpelloid stamen. The carpelloid stamen bears ovules on them. In CMS rice, variations in the timing of tapetal break down occur in different locules of same anther (Agadi, 1996).

Variations in the break down time of microsporogenesis in five different plasma types of *Petunia hybrida* are attributed to their generic variations
(Izhar and Frankel, 1976). In all the five plasma types, the first sign of breakdown of microsporogenesis is at early prophase in the form of changes in free amino acid composition. According to Izhar (1977), variations in the breakdown time of the microsporogenesis are due to interaction of the plasmagene with temperature-sensitive genes that control the breakdown time. 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 are 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 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.

Alterations in the temporal synthesis/dissolution of callose appear to have a significant role in generating male sterility. 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). Persistent 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 (Graybosch and Palmer, 1985a, 1987), CMS mutant of Glycine max (Wei, Palmer and Horner, 1996), Brassica napus. (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 supposed to be due to the malfunctioning of the tapetum. 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 male fertile 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, premature callase activity correlates with lower pH (Izhar and Frankel, 1971). In Lilium, chilling delays the acidification of anther locule medium and suppresses the callase activity at proper time (Koike, 1997). According to Wei et al. (1996), in male sterile Glycine max, reduction in callase mRNA suggests abnormal transcription.

In male sterile mutant of Oenothera, alterations in the chemical composition of callose lead to male sterility (Noher de Hallac 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.

The contention that the premature callose dissolution leads to collapse of the developing microspores (Izhar and Frankel, 1971) has been confirmed in transgenic tobacco anthers (Worrall et al., 1992). Abnormal pollen wall development in this plant is presumed to be due to absence of callose.

Cytochemistry (histochemistry) of male sterile anthers.

Often, the structural and chemical aberrations of anther tissues complement each other. The nutritional imbalances in male sterile anthers stem
from several sources. In CMS Beta vulgaris (Rohrbach, 1965), Triticum (Joppa et al., 1966), Sorghum (Alam and Sandal, 1967) and Oryza (Agadi, 1996) stamens show poorly developed vasculature. In two GMS lines of rye (Cebrat and Zadecka, 1978) vascular bundle of the stamens is surrounded by endoderm-like suberized cells. A poor or abnormal development of vasculature is believed to impede the transport of nutrients, thereby resulting in an inadequate accumulation of nutrients in the anther tissues. Autoradiographic studies on CMS sugar beet have shown relatively more accumulation of $^{14}$C compounds in the tapetum at tetrad stage (Nakashima and Hosokawa, 1971). The connective of sterile anthers possesses 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). The possibility of hormonal imbalance causing defective nutritional condition is also suggested (Cheng et al., 1979).

Fertile and sterile anthers showing quantitative differences in nutrients have been reported in other species also. In Sorghum and Zea mays both male sterile and fertile anthers, during their early developmental stages, 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) report persistence of starch in the wall layers and connective of CMS sunflower. This is attributed to the persistence of callose around tetrads (Hegde and Isaacs, 1992). Subsequently the nutrients accumulated in wall layers are resorbed causing the enlargement of the tapetum and middle layer. Male sterile rice anthers also show a link between rich accumulation of non-reducing sugars and starch, coupled with low inorganic phosphate and acid phosphatase activity in tapetum and degeneration of microspores (Nishiyama, 1984). The tapetum becomes dilated with condensed cytoplasm accompanied by augmentation of mitochondria, proplastids, 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.

Male sterile anthers of Cucumis melo show low activities of succinic dehydrogenase (Fukasawa, 1961) and acid phosphatase (Chauhan and Singh, 1968). Low amylase and high polyphenol oxidase and peroxidase activities are reported in the male sterile anthers of maize (Dmitrieva and Khavzhinskayia, 1962). In male sterile stamenless2 mutant of tomato low esterase activity correlates with delayed degeneration of tapetum and failure of exine deposition (Sawhney and Bhadula, 1987).

A comparative composition of free amino acids in male sterile and fertile lines has been analyzed in 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

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(Ogura, 1968) this amino acid is totally absent. Male sterile anthers show more alanine (Hosokawa et al., 1963) and aspartic acid (Rohrbach, 1965) in Beta vulgaris and glycine in Sorghum vulgare (Brooks, 1962). Male sterile lines of Sorghum vulgare Var. Sudanese contain low quantities of alanine, glutamic acid, proline, phenylealanine and tyrosine (Alam and Sandal, 1972). Tripathi et al. (1981) report low histidine, thieonine, glutamic acid, leucine and phenylalanine, but higher concentration of alanine, serine, proline and tyrosine in male sterile Sorghum. According to Tripathi et al. (1981) the difference in the amino acid composition in the anthers of sterile and fertile lines is suggestive of its involvement in growth and development. According to Izhar and Frankel (1973) amino acid imbalance probably induces changes in pH of the anther locule.

In the anthers of CMS barley, abnormality is expressed in the form of uncontrolled secretion of sporopollenin (Ahokas, 1978). It is envisaged that the 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 lobe collapses and exine of microspores fuses with the locular sporopollenin and locular wall.
Absence of endothecial fibrous wall 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 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 anthers tips fibrous thickenings are developed. In the sterile corn anthers despite absence of endothecial thickenings endothecial 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 in 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 mobilization of copper at critical stage of microsporogenesis.

Tian et al. (1998) report abundant calcium in the anther walls, tapetum, surface of pollen grains and ubisch bodies of the fertile anthers and only in the middle layer and endothecium GMS rice. A special wall formed between the tapetum and middle layer of sterile anthers accounts for specific calcium accumulation patterns and poor pollen wall formation (Tian et al., 1998). The anomalies in the distribution of calcium accumulation are correlated with the failure of pollen development and pollen abortion.

CMS Petunia anthers differ from fertile and restorer anthers in having relatively low esterase activity (van Marrewizk 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 the 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 differences in the esterases and cytochrome oxidase activity are effects rather than a cause for the pollen sterility in Petunia hybrida.

Anthers of male sterile maize show reduced activity of cytochrome oxidase from pre-meiosis onward (Bino et al., 1986). Later, the mitochondrial localization of cytochrome oxidase in fertile pollen confines to cristae and within the space between the outer and inner limiting membranes of the organelles. In the 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. In CMS sunflower also, prior to visible structural deviations, sporogenous cells possess low activities of succinic dehydrogenase, cytochrome oxidase and malate dehydrogenase (Hegde and Isaacs, 1992).

Comparative analyses of the activity of amylase and level of starch and soluble sugar contents in the normal, 'gibberellin-sensitive', and GA₃-reverted mutant stamens of tomato (Bhadula and Sawhney, 1989) have shown that the sterile stamens, during post meiotic phase, possess significantly low amylolytic activity. At maturity starch content decreases in normal anthers while storage persists in mutant stamens. The mutant stamens also contain low levels of soluble sugars. In GA₃-treated (Sawhney and Greyson, 1973b) and GA₃-reverted mutant stamens (Bhadula and Sawhney, 1989), the amylolytic activity and the level of starch and soluble sugars are comparable to normal stamens. According to Bhadula and Sawhney (1989) low levels of endogenous gibberellin in sterile stamens affect the activity of amylases. Reduced activity of amylase results in low sugar levels, which ultimately leads to abnormal pollen development. In a parthenocarpic mutant of tomato also GA₃ treatments are found effective in restoring carpelloid anthers to the wild type phenotypic (Mazzucato et al., 1999).

According to Kaul and Sudha (1990) reduced biochemical components are regular features of male sterile anthers. They report relatively low carbohydrate and protein metabolism in the mutant anthers of barley. 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.

Estimation of free putrescine, spermidine and spermine levels and the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase in the floral organs of the normal and male sterile stamenless2 mutant of tomato have shown that the abnormal stamen development is, in part, related to high levels of endogenous polyamines (Rastogi and Sawhney, 1990a). Rastogi and Sawhney (1990b) could induce abnormal stamen development in polyamine treated normal flower and normal-looking pollen in polyamine-inhibitor treated mutant flower buds.

The requirement of benzylaminopurine and gibberellic acid, in addition to 4-5% sucrose, for in vitro growth of flower buds of male sterile stamenless2 mutant of tomato suggests that the mutant flower buds contain low levels of endogenous gibberellins (Sawhney and Rastogi, 1990). Since gibberellins induce the synthesis of amylases, which in turn are required for the break down of starch to provide free sugar for the developing organs, the low levels of gibberellins present in the mutant bud cause abnormal development of pollen grains through inadequate production of soluble sugars (Sawhney and Rastogi, 1990).

Comparative cytochemical analyses of anthers of wild and nuclear male-sterile mutant of Arabidopsis thaliana have shown that in mutants microspores show reduced RNA, alcohol dehydrogenase and esterases, incomplete intine, late vacuolation and mitosis and lack of adenine
phosphoribosyl transferase (APRT) (Regan and Moffatt, 1990). During development of the normal anther free adenine, produced from the breakdown of the purine nucleotides and nucleotide factors, is rapidly recovered or saved by APRT. In the mutant stamens, adenine is not saved because of absence of 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 tissues caused by 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.

Report from Singh et al. (1992) reveals that stamens of normal and male sterile stamenless2 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) temperatures the mutant leaves contain 10-20 times higher IAA concentrations than the normal leaves. At intermediate and high temperature, 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).
In CMS *Brassica napus* (Shukla and Sawhney, 1992) the leaves contain highest levels of cytokinins as compared to the other organs. The normal lines show higher levels of cytokinins in the root, stem and mature flowers. These authors envisage that the lower levels of cytokinins in the flower of CMS line of *Brassica napus* are likely involved in the expression of male sterility.

**Molecular events of male sterility.**

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, 1983; 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) envisage a relationship between
polymorphism in mitochondrial genes and ATP synthase complex. They propose that mitochondrial gene products are incompatible with the complementary products produced by the nuclear genome. Compatibility depends upon presence of complementary 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).

The CMS system in common bean differs from other well defined CMS systems where the phenotype arises as a consequence of dominant mutations in the mitochondrial genome (Sarria et al., 1998). In this plant CMS-associated mitochondrial mutation (prs-orf 239) is transcribed in both vegetative (young seedling) and reproductive tissues. However, its product (ORF 239) is present only in reproductive tissues. The ORF 239 product ultimately is localized within the callose layer of the pre-meiotic meiocyte wall. According to Sarria et al. (1998) plant mitochondria in vegetative tissues contain a LON-like protease (present in E. coli), which causes the degradation of the mitochondrial sterility-associated mutant protein ORF 239. During the development of meiocytes in sterile anther the ORF 239 protein is apparently stable, accumulating within the callose layer and the primary cell wall. However, it is not clear whether the LON protease is expressed or active in the meiocytes.

In male sterile oil seed rape, the fertility is restored by a chimaric ribonuclease-inhibitor gene (Mariani et al., 1992). A cross between male sterile plant expressing a chimaric ribonuclease genes in the anther tapetal cells and male fertile plants that was transformed with a chimaric tapetal cells specific
ribonulease-inhibitor gene produced fertile F1 plant by the suppression of cytotoxin ribonuclease RNase/RNase inhibitor complexes.

In transgenic Petunia inhibitor of pigment synthesis causes male sterility (van der Meer et al., 1992). This implies 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).

In male sterile chicory plants, generated by the fusion of chicory mesophyll protoplasts and male sterile sunflower hypocotyle protoplasts, the mitochondrial DNA is contributed from the 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 rearrangements 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 sunflower gene (Kohler et al., 1991; Laver et al., 1991) or whether the fusion process has given rise to a new chimaric 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 is partly due to non-disruption of other essential mitochondrial genes by CMS associated mutant mitochondrial genome (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 tissues than in vegetative and female reproductive tissues and that mutant mitochondria in the anthers of CMS lines can not support their higher demands.

Levings (1993) and Smart et al. (1994) provide explanation for tissue specificity of the mutant 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,
possibly, because high levels mitochondrial gene expression and biogenesis are 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 becoming a male gametophyte, existing independent of the parent anther tissue. The mitochondrial mutation may disrupt or impair mitochondrial biogenesis in the meiocytes leading to cell abortion.

Recent study by Prymakowska-Bosak et al. (1999) reveals the role played by the linker histones in male meiosis and pollen development. In transgenic tobacco decrease in the major variants of linker histones HI A and HI B is compensated by the increase in the minor variants of linker histones HI C, HI D, HI E and HI F. Plants with deficient HI A and HI B linker histones showed aberrations in flower development and were completely male sterile. These features correlated with changes in the temporal, but not the spatial, pattern of expression of developmental genes. The aberrations in male gametogenesis resulted from disturbance in correct pairing or segregation of homologous chromosomes during meiosis.