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Inability of the plant to produce functional pollen leads to male sterility in higher plants. By crossing two lines of desired characters, the plant breeders are able to obtain F1-hybrids which exhibit hybrid vigour or heterosis. However, in order to avoid self pollination, the anthers from the female parent have to be emasculated. In a country like India where the availability of labour force made it possible to remove anthers from sibbing, however, this process is tedious and labourious, and the cost of the resultant hybrid is too high. Since male sterile lines circumvent the costly procedure of hand emasculation, the use of male sterile lines has revolutionized the conventional plant breeding programmes in the production of hybrid seeds.

Male sterility in plants is exhibited in diverse forms like absence of normal anthers, reduction in anther size, varying degree of petaloidy, abnormal meiosis followed by formation of empty shrivelled microspores, normal meiosis followed by abnormal development of microspores, and normal meiosis accompanied by failure of athesis in which case pollen is not shed at all (Kaul 1988). There are two types of male sterility in higher plants Viz. (1) Genic male sterility (GMS) and (2) Cytoplasmic male sterility (CMS). The former is due to nuclear genes while the latter is caused by incompatibility of mitochondrial nuclei. Since GMS is nuclear, it follows mendelian pattern of inheritance, which makes its utilization difficult because the resultant progeny is 50% fertile and 50% sterile. One the other hand 100% sterility is achieved in CMS lines. The latter approach has prompted the plant breeders to go in for CMS lines for hybrid production.

The involvement of cytogenes in controlling wide range of vegetative and floral abnormalities (Harvey et al., 1972; Tsunewaki 1980 b), female sterility (Grun 1976), disease susceptibility (Hooker 1974), herbicide tolerance (Machado et al., 1978), heterosis (Srivastava 1981), tentoxin sensitivity (Durbin and Uchytil 1977; Flick and Evans 1982) and antibiotic sensitivity (Menczel et al., 1983; Kinoshita and Mikami 1984; Medoyesy et al., 1985), has been well studied in plants. Of all
these anomalies—excluding female sterility which has negative- breeding value, male sterility with its excellent genetic potential has provided an exceptional opportunity to dissect out independent cytoplasmic and nuclear genome functions and interactions (Leaver and Gray 1982; Hanson and Conde 1985; Kaul 1988).

Cytoplasmic male sterility (CMS) is widespread in occurrence in higher plants. It is reported in 342 species and species crosses (Kaul 1988). The majority of CMS are due to spontaneous as well as intraspecific and intrageneric hybridizations. CMS can also be induced by various chemical or physical mutagens and is reported in beet, pearl millet and grass pea (Kaul 1988). Environmental factors like temperature and photoperiod also influence the expression of CMS (Kaul 1988). CMS, being extrachromosomal in inheritance, transmits its expression to the whole progeny, through the male gamete which generally contributes a very small amount of cytoplasm to the zygote. However, in many cases of CMS, parental genotype may totally or partially restore the male fertility expression of the progeny through CMS seed line. However, the nuclear background is also known to influence the phenotypic expression of cytoplasmic genes. Monogenic and polygenic restorer systems are known in this regard, and recessive and dominant sterility modifiers have been identified (Hanson and Code 1985).

The involvement of mitochondria in cytoplasmic male sterility has been well documented (Hanson 1991). Restriction Fragment Length Polymorphism (RFLP) of mitochondrial DNA reveals that the male sterile gene is responsible for CMS - e.g. urf gene in CMS T maize, Pcf in Petunia and Cox I in Sorghum. These genes encode certain specific polypeptides which may be involved in pollen abortion. In maize CMS, T urf encodes 13 KD Protein (Levings 1990). While Pcf gene in Petunia encodes 25 KD protein (Nivision and Hanson 1989), Cox I in Sorghum encodes 42 KD protein (Dixon and Leavvar 1982). Similar variation in translation products from mutant mitochondria is also found in Vicia faba (Boutry and Briquet 1982), Beta vulgaris (Powlung and Ellis 1983), Nicotiana tabaccum
These specific polypeptides may be involved in pollen abortion in CMS plants as suggested in the work of Koler et al. (1991). Although they fused the male sterile cultivar of *Nicotiana* having a common nuclear origin, cytoplasm in these are from different species. The plants regenerated from cybrids exhibit a wide variety of phenotypes. These include a novel phenotype which is unlike that of either parents or homeotic transformation of stamen tissue to petal-like or carpel-like tissue. Analysis of mt-DNA of male sterile cybrids and their parents reveals that the mitochondrial DNA of male sterile cybrids with parental floral morphology is unchanged when compared with parental mt-DNA. Interestingly, while the cybrids are morphologically similar to one of the parents, male sterile phenotype has mt DNA almost identical to that of parent. However, cybrids with recombined biparental or novel male sterile phenotypes contain mt DNA different from both male sterile parents and also from each other. These observations led the authors to suggest that change in mt-DNA are always associated with changes in floral phenotype, and these changes are predictive of changes in mt DNA. These instances indicate that there are two separate mitochondrial genes involved in petal development, and at least one of them cause stamen formation.

The consequences of male sterile gene action on anther development varies from one line to another. It may act either prior to or during pre-meiosis and post-meiosis. Ironically, most of the abnormalities in CMS plants are reported in tapetum. The tapetum which is being the innermost wall layer of anther, is involved in as many as ten functions, mostly associated with the nourishment of meiocytes, production and release of callase, and formation of pollen wall components (Pacini *et al.*, 1985). It is reported that the tapetal development and the course of microsporogenesis and its breakdown stages are known only in 12% of the CMS plants documented. Of them, in 16% the tapetum is normal, in 35% abnormal and in 49% it is persistent. However, the breakdown of microsporegenesis is observed in all the cases (Kaul 1988). To illustrate this
point, certain examples of crop plants in which abortion of microsporogenesis takes place in brief as follows:

In *Capsicum annuum*, Horner and Rogers (1974) reported normal pollen development up to microspore formation in both CMS and fertile lines. However, in CMS anther before and during meiosis the tapetal cells are enlarged and highly vacuolated i.e. hypertrophied. These tapetal cells remain oppressed to the meiocytes and thereby no locular cavity formation takes place resulting in the degeneration of microspore tetrads. Similar observation has also been made in *Capsicum annuum* by Manoharan (1992) who, however observed that tapetum in both CMS and fertile lines become very large and conspicuous, and hence invalidated the hypertrophied nature of tapetum in CMS anther. In *Helianthus annuus* (Horner 1977) degeneration and disintegration of CMS tapetum and microspore tetrads occur after meiosis II resulting in sterility. In *vicia faba* (Audran and Willemse 1982) based on fluorescence study, it was found that the sterile pollen posses relatively thick endexine and no intine.

In CMS C maize, deviation from normal pollen development was observed in tapetal cells at the tetrad stage of development (Lee et al., 1979). In CMS S maize, pollen abortion begins in nearly mature pollen grains, its tapetum being normal (Lee et al., 1980). In CMS T maize, the breakdown of tapetal mitochondria and mid- anther layers are the first visible signs of anomalies that occur shortly after meiosis (Warmke and Lee 1977).

The finding of Colhoun and Steer (1981) using light microscope agrees with that of Warmke and Lee (1977) and Lee et al. (1979). In *Secale cereale* (Seolec and Evans 1979) Pollen degeneration is post - meiotic. The tapetum becomes vacuolated and invades the anther locule. This is followed by conversion of microspores, tapetum and middle wall layer into unorganized mass within the locule. In *Petunia hybrida* Bino (1985) reported that microsporogenesis in sterile anthers proceeds normally until leptotene. At this stage, the first sign of aberrant development manifests in tapetum which subsequently begins to degenerate along with the meiocytes. In *Crotalaria*
pallida (Arora and Gupta 1984), the tetrads are not formed in CMS anthers. It is suggested that the sterility is caused due to differences in tapetal behaviour and failure of cytokinesis in sterile anther.

In normal and male sterile stamenless-2 mutant tomato (Sahawnay and Bhadula 1988), no significant differences were observed until the formation of tetrads. During the release of microspores from the tetrad, tepetal cells in both lines become amoeboid and showed sporopollenin like deposits in the tapetum. Subsequently, the cells of the normal line degenerate, while those of mutant remain intact and vacuolate. No exine deposition was observed in mutant microspores which enlarge and eventually degenerate. The degeneration of the mutant tapetum occurs at very late stage in the anther development.

In CMS Citrus, archesporial degeneration has been observed in the CMS anthers (Upholt 1931; Nakumura 1934, Krug and Bacchi 1943) and consequently no meiosis ensues. In Nicotiana (Burk 1960), the staminal transformation produces either malformed petal-like structures or petals that have distinct stigma-tipped extensions. In majority of Nicotiana tabacum plants exhibit staminal feminization, and petaloidy is exhibited. But, in a few, this transformation does not occur. In their anthers, sporogenous tissue develops abnormally and no pollen is produced. In CMS Daucus carota (Thompson 1961), no meiotic traces, and post prophase-I have been detected. In CMS cotton PMC's degenerate before meiotic initiation (Murthi and Weaver 1974) which is followed by tapetal disorganisation.

Endothecium and vascular strands of the anther are also under male sterile gene attack, and thereby influencing pollen abortion (Laser and Lersten 1972).

As the concept of involvement of mitochondria in male sterility gains momentum, many workers have attempted to elucidate the cellular and sub-cellular events, especially structural changes in mitochondria. In CMS wheat, De Vries and Ie (1970) found no cytoplasmic differences. In Sorghum Overman and Warmke (1972) reported cytoplasmic disorganization during degeneration of tapetal layers. In
Capsicum, Horner and Rogers (1974) and Manoharan (1992) could not find any evidence of organelles disruption. Turbin et al., (1974) in CMS wheat showed a reduction in the number of large organelles in the cytoplasm of premeiotic pollen mother cells. In Sugarbeet, Nakashinma (1975) implicated the abnormal behaviour of tapetal organelles for abortion of developing microspores. In Sunflower, Horner (1977) described the disarrangement of organelles concomitant with the enlargement of tapetal cells. Warmke and Lee (1977) observed the mitochondrial abnormality in the form of early breakdown of tapetum and middle layer in the sterile anthers of CMS T maize. Again, Lee and Warmke (1979) reported a rapid increase of 20 to 40 fold in mitochondrial number per cell preceding tapetal breakdown in sterile anthers. Extending their studies further to CMS-C Maize lines, Lee et al. (1980) could not find any organellar changes. Working on the same line in which Warmke and Lee (1977), and Lee and Warmke (1979) studied the CMS T cytoplasm of maize, Colhoun and steer (1981) described the presence of apparently intact mitochondria in the tapetal cells.

Bino (1985 b) observed the presence of large vacuoles in the cytoplasm of tapetal cells as an initial aberration and he could not find organellar differences in Petunia. Li and Chu (1987) described several mitochondrial anomalies ranging from mitochondrial swelling to reduction of cristae and loss of matrix in rice. However, Laveau et al. (1989) found no abnormality in organelles of sunflower. In Brassica where the stamen morphology is influenced by temperature conditions, Polowick and Shawney (1990) observed changes in mitochondrial matrix and criste. No mitochondrial abnormality has been reported in the anthers of CMS-s onion (Holford et al., 1991). Since most of the alternations or deviations in the anther development of CMS line are accompanied with biochemical changes, several investigators have focussed their attention on the analysis of amino acids and proteins in male sterile and fertile anthers.

Analysis of free amino acids by Fukasawa (1954) revealed that CMS T
maize anthers have comparatively less amount of proline and more amount of asparagine than that of fertile anthers. The absence of proline was reported by Hosokawa et al. (1963) in the anthers of male-sterile beet.

Khoo and Stinson (1957) could not detect proline in the anthers of a CMS maize. Similarly proline was not detected in the sterile anthers of radish (Ogura 1968). The CMS-T corn anthers also accumulated alanine precociously at tetrad stage whereas in normal fertile anthers, alanine accumulation took place just before anthesis (Khoo and Stinson 1957). Nakashima and Hosokawa (1970) reported the presence of proline in the mature fertile anthers, but not in CMS anthers of Corn and Sorghum.

In Sorghum vulgare (Brooks 1962), male fertile anther accumulates more glycine than in sterile anthers. In Sorghum vulgare var. sudanese (Alam and Sandal 1972), no significant differences in free amino acids occurred between sterile and fertile anthers of pre-meiotic stages. However, during free pollen stage, alanine, glutamic acid, proline, phenylalanine and tyrosine are lower in sterile anthers. In the pollen grains of seven diploid (sterile) and seven triploid (sterile) varieties of apple (Tupy 1963), more proline and less histidine were observed in the fertile pollen grains than in the sterile pollen grains, whereas in sugarbeet (Hosokawa et al., 1963) no significant differences were observed in the content of other amino acids between sterile and fertile anthers. The paper chromatographic results showed a large amount of alanine, but absence of proline in the sterile anthers at all stages of anther development.

Rohrbach (1965) reported increase in the aspartic acid in the developing sterile anthers, but a decrease of it in the fertile anthers. Similarly, an increase in asparagine content is reported in Petunia (Izhar and Frankel 1973).

Electrophoretic studies of fertile and sterile anthers reveal differences in their protein profile. Markova and Daskaloff (1976) reported 15 and 17 protein bands, respectively in CMS and normal lines of Capsicum. However, by using SDS PAGE, Manoharan et al. (1993) observed the absence of 20 KD protein during
pollen mother cells formation and subsequent stages of abortion. In *Sorghum*,
Alam and Sandal (1969) reported 20 bands in fertile and 9 bands in sterile lines.
In *Petunia* (We and Murray 1985), the absence of 63 and 43 KD proteins is
reported during meiosis where pollen abortion begins in sterile line. 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. Similarly, electrophoretic analysis has also revealed consistently fewer
bands of peroxidase and cytochrome oxidase at pre-pollen stages in *Sorghum*
(Alam and Sandal 1969). Esterase isoenzyme patterns of male fertile and CMS
wheat (Hohler and Borner 1980) showed two isoenzyme bands which were missing
in male sterile anthers. These missing bands were correlated with the absence of
sporopollenin hydrolysing esterase isoenzyme in sterile anthers. A comparative
account of esterase activity and its composition in male fertile, restored male-
fertile and CMS anthers of *Petunia hybrida* (Van Marrewijk et al., 1986) denote
similar isoenzyme patterns in the male fertile and restorer idotypes till the tetrad
stage. In CMS anthers esterase activity remained low without any new isoenzyme
bands during pollen abortion. Deficient cytochrome oxidase system is observed in
maize (Watson et al., 1977) and rice (Dai et al., 1978). Loseva et al. (1974) found
a lower intensity of oxidative phosphorylation in mitochondria of sterile anthers
and an increase in ATPase which led to lower ATP content in maize. Similarly, in
*Petunia*, Liu et al. (1988) observed efficient export of ATP from mitochondria of
fertile than from the mitochondria of CMS plants. Fewer banding patterns and
activities of malate and succinate dehydrogenases were observed in wheat
(Baidulova Babko 1983), Corn (Ohmura et al., 1976) and maize (Palilova et al.,
1977) and glutamate dehydrogenase in maize (Loseva and Mikulich 1979) are all
found in sterile anthers. In contrast, increase in cytochrome oxidase activity is
reported in maize (Markova 1983).

Very few histochemical studies have been carried out in the anthers of
CMS and fertile lines. In the male fertile and sterile anthers of sugarbeet,
Hosokawa et al. (1963) have reported accumulation of starch grains in the young
anthers. In fertile anthers, a decrease in starch content is correlated with the progress in anther development. In the sterile anther, starch grains persist throughout the anther development. Sugars were reported in the early stages of anther development in the fertile ones, but not in the sterile anther. In wheat (Anonymous 1976), both male fertile and sterile anthers showed starch accumulation in endothecial cells at early stages. However, at anthesis, starch was lost in sterile anthers of endothecium. The pollen of fertile anthers was richly endowed with starch storage. Histochemical and micro autoradiographic studies were conducted in CMS sugarbeet, Corn and Sorghum by Nakashima and Hosokawa (1970, 1971, 1974 b) and Nakashima (1975). In corn and Sorghum, reducing sugars gradually increased in both fertile and sterile lines. The endothecium and connective of fertile anthers in corn and Sorghums show starch storage, but not in the CMS lines of anthers. In fertile and genic male sterile sunflower anthers, Nakashima and Hosokawa (1974 a) found no differences in the distribution of DNA, polysaccharides and proteins. In Zea mays. Cheng et al. (1979) showed the deposition of starch in the endothecium and epidermis at pre-meiotic stages in both sterile and fertile anthers. In the former, starch accumulation was lost subsequent to cytoplasmic disorganization in the tapetal cells at young microspores stage where pollen abortion takes place. In the fertile anthers starch accumulation persisted even at the pollen grain stage. Chauhan and Singh (1968) in Cucumis melo noticed a gradual increase in the acid phosphatase activity in fertile anthers until late tetrad stage, after which the activity of this enzyme decreases and tapetum degenerates during microspore development. On the contrary, in the sterile anthers the activity of this enzyme is considerably low. Similar histochemical localization of acid phosphatase during different developmental stages in CMS and fertile lines of Allium cepa, Capsicum annuum, Cucurbita marina, Datura alba, Solanum melongena and Triticum aestivum shows less activity in sterile anthers.

In Petunia (Bino et al., 1986) a decline in cytochrome C oxidase activity was observed in sterile anthers from meiosis stage onwards when initial symptoms
of tapetal degeneration were already apparent. In addition, in fertile anthers cytochrome-C oxidase was localized in the cristae and also within the space between the outer and inner limiting membranes of the mitochondria, whereas in CMS anthers cytochrome-C oxidase activity was recorded only between the outer and inner membranes. These differences occurred at stages when no structural signs of degeneration were apparent. The lack of sufficient transport of nutrients due to poorly developed vascular bundles in the stamens of male sterile plants in *Triticum* (Joppa et al., 1986) and *Sorghum vulgare* Var. *sudanense* (Alam and Sandal 1967 a, 1967 b) has been correlated with low accumulation of starch in the tapetal cells. Using autoradiographic studies Nakashima and Hosokawa (1974 b) have shown that CMS sugarbeet and corn spikelets fail to translocate photosynthates into the anthers. In sugarbeet $^{14}$C assimilates were found in the tapetum and tetrads of both fertile and CMS lines. But later, labelled carbon was seen blocked in the tapetum of CMS-line. In fertile anther labelled carbon was observed in the microspores. The male sterile gene also affects the enzyme callase which is synthesized from tapetum and involved in the degradation of callose layer surrounding the microspore tetrads. Delayed or precocious activity of callase disturbs microsporogenesis, and lead to pollen abortion in *Petunia* (Frankel et al., 1969; Izhar and Frankel 1971), *Gossypium* (Manoharan et al., unpublished), *Lolium* (Hayward and Manthiraratna 1979l) *Allium* (Nanda and Gupta 1974), *Sorghum* (Warmke and Overman 1972), *Phaseolus* (Pritchard and Hutton 1972) and *Pisum* (Gottschalk and Kaul 1974).

**Aim and Scope of the present study**

The anther of angiosperms, a target site for male sterile gene action, represents a complex system in which the gametophytic and sporophytic generations of the plants communicate, co-ordinate and apparently co-participate in the anther development (Ursin *et al.*, 1989). The anther is composed of several different tissues comprising heterogenous cells and tissue types, and hence a large
number of genes are required to programme the entire process of pollen development. It is estimated that about 15,000 different genes are involved in the developing tobacco anther and several of the corresponding mRNAs are spatially restricted to the innermost layer of the anther wall, the tapetum (Vergne and Dumas 1988). Not surprisingly, the initial abnormality in many a CMS systems has been reported in tapetum (Kaul 1988).

During the past few years, the structural, developmental and physiological aspects of the anthers development and of normal and male sterile lines of crop plants have received considerable attention. This has been evidenced by the ever accumulating literature (Kaul 1988). In the majority of plant species however, biochemical differences between sterile and fertile lines of anthers have not been investigated. The limited studies available comparing some biochemical components and enzyme levels of male sterile and fertile anthers in some plants revealed reduction in (a) carbohydrate, ascorbic acid and protein levels (b) total nucleic acid contents and (c) enzymatic activities of dehydrogenases, peroxidases, esterases and phosphatases etc. These studies were conducted principally by extracting the metabolites from the anthers. But, as mentioned earlier, it is necessary to take into account the fact that, the anther of angiosperms is an integrated biological system of highly specialized and heterogenous tissues. Any biochemical change that occur in the individual tissue of anther, in all possibility, affect the other constituent tissue, and the fact that each of which performs certain specific function during the course of microsporogenesis, should be looked into. In this respect, in situ investigations of macromolecular substances namely carbohydrates, ascorbic acid, RNA, DNA, proteins, and enzymes systems, are a necessary pre-requisite for establishing the prevalent interactions among the tissues in the anther, which provide conditions for pollen development. Therefore, in the present investigation the following aspects were considered:

1. Histological aspects of microsporogenesis in fertile and sterile lines for understanding precisely, when and where male sterility initiates and its
2. Localization of callose in fertile and sterile anthers, as the premature or delayed dissolution of callose around meiocytes and microspore tetrads has been implicated in many CMS taxa (Kaul 1988).

3. *In situ* localization of some histochemical substances namely, carbohydrates, ascorbic acid, nuclei acids (RNA and DNA) and proteins in the fixed materials.

4. Localization of enzymes namely Glucose-6-phosphate dehydrogenase, Succinate dehydrogenase, Malate dehydrogenase, Acid phosphatase, Alkaline phosphatase, ATPase, Non-specific esterases, Peroxidases and Cytochrome oxidases, using fresh cryostat-sections. Attention has also been paid to localize lipids. All these macromolecular substances-enzymes, have been studied at successive developmental stages of anthers development of both sterile and fertile lines.

Attention has been paid to those mitochondrial enzymes which are involved in respiration. These enzymes have been localized with the premise that any abnormality in the mitochondria may reflect on the synthesis of various dehydrogenases. We are particularly interested to find out any specific enzyme(s) affected in sterile anthers.