CHAPTER V
GENERAL DISCUSSION
Understanding the mechanism of male sterility in higher plants is very essential for its application in the production of hybrid seeds. Male sterility in plants has diverse manifestations, including absence of male sex organs, union of stamens with gynoecium, reduction in anther size, atrophy of anthers, varying degree of petaloidy, reduction of the stamens into staminodia or conversion of the male sex organ into carpels, abnormal meiosis, collapse of microspores, normal meiosis followed by abnormal development of microspores, and normal meiosis followed by indehiscent anther formation (Van Marrewijk 1979; Kaul 1988). The most common type is pollen sterility. It is widely spread throughout the plant kingdom. Male sterility is defined as the incapacity of plants to produce or to release functional pollen. Male sterility is usually controlled by hereditary factors and in most cases governed by one nuclear gene. In some members pollen sterility is brought about by extra nuclear hereditary factors or by the joint action of both nuclear genes and extra chromosomal factors: Cytoplasmic male sterility (CMS). CMS primarily expresses itself by deterioration of the sporogenous tissue. The time of collapse ranges from premeiosis to the maturing pollen stage. Generally, CMS is accompanied by other histological and biochemical abnormalities in the male sex organ.

The data in the literature however cause confusion, because the different aspects have been investigated separately in different stages of development for various crops of economically important under varying conditions. The present discussion is aimed at obtaining some insight in the mechanism of CMS to discover where the process of the deregulation in the sporogenous development takes a start during microsporogenesis in the anther.
In the present study an attempt has been made to correlate developmental and histological data with biochemical and molecular aberrations in CMS cotton. Several previous studies have revealed that the genetic determinants for CMS in higher plants are carried by mitochondrial genome (Levings and Pring 1976; Levings 1983; Mikami et al 1984; Hanson and Conde 1985; Lonsdale 1987; Young and Hanson 1987; Turpen et al 1988; Hanson 1991; Nair 1993; Krishnasamy and Makaroff 1994). We observed that in CMS cotton, mitochondria synthesize two additional polypeptides of 36 kd and 48 kd. Whereas CMS anthers of Petunia lack 63 kd and 45 kd proteins (Wu and Murry 1985). In CMS Capsicum (Manoharan et al 1993) and CMS maize-T, mitochondria (Levings 1990) synthesize 20 kd and 13 kd polypeptides, respectively which are absent in normal lines. CMS Sorghum mitochondria lack 38 kd protein (Dixon and Leaver 1982). In cotton, it is hypothesized that these additional polypeptides are responsible for the pre mature abortion of meiocytes in the CMS anther, and that they might inhibit the synthesis of proteins necessary for normal pollen development. Variations in mitochondrial translation products have also been reported in other normal and male sterile systems (Boutry and Briquet 1982; Powling and Ellis 19983; Boutry et al 1984; Hakansson et al 1988; Krishnasamy and Makaroff 1994). In a few systems chloroplasts have been implicated in causing CMS. In cotton, chloroplast DNA encoded protein differences between fertile and sterile lines have been reported (Chen and Meyer 1979).

In CMS plants aberrant mitochondria have been correlated with several structural and functional deviations in different anther tissues, including sporogenous cells, tapetum, and other wall layers. These mitochondrial mutations and/or nuclear mitochondrial incompatibilities associated with CMS plants result in the disruption of normal pollen formation, but do not affect female fertility (Hanson and Conde 1985).
CMS also provides one of the few systems in which nuclear-mitochondrial interactions and their role in plant development can be investigated. CMS therefore, has two-fold value. 1) It is potentially useful in hybrid seed production and, 2) it is valuable in investigating the gene controlled mechanisms in stamen development (see McCormick et al. 1991, McCormick 1993; Davies et al. 1992; Mascarenhas 1992, Zaki and Dickinson 1992 and references therein). The involvement of tapetum in microsporogenesis is well documented (Bhandari 1984; Shivanna and Johri 1985; Pacini et al. 1985; Rudramuniyappa and Manure 1993). Abnormalities of various kinds in tapetal functions may lead to pollen sterility in higher plants (Mascarenhas 1975; Bhandari 1984; Shivanna and Johri 1985; Pacini et al. 1985; Theis and Robbelen 1990). In CMS cotton the tapetum does not show any structural or developmental abnormality during abortion of microsporocytes. Further sub-cellular observations of tapetal tissue during microsporogenesis in CMS anther are essential to determine if there are abnormalities in organelle structure and distribution. Histochemical studies on CMS and fertile anther during microsporogenesis in cotton show a difference in distribution of insoluble polysacharides and protein content. We interpret that this difference underlines the importance of the tapetum in nourishment of microsporocytes during microsporogenesis.

In normal microsporogenesis the role of callose has been very well documented (Bhandari 1984; Rudramuniyappa and Manure 1993, and references therein). Proper timing of the build-up (around the microsporocytes) and degradation (after meiosis) of callose appears to be very essential for the production of viable pollen grains. Degradation is brought about by enzyme callase (β-1,3-glucanase) (Frankel et al. 1969). Many observations have indicated differences in callose behaviour of microsporocytes in fertile and sterile plants (Izhar and Frankel 1971; Warmke and Overman 1972; Stelly and Palmer 1982; Theis and Robbelen 1990). Remarkable
difference in enzyme callase activity has been studied in Petunia (Frankel et al 1969; Izhar and Frankel 1971; Van Marrewijk and Suurs 1985; see Kaul 1988 and references therein). Abnormal behaviour of callose wall in microsporocytes is undoubtedly related to CMS. Recently, the involvement of enzyme callase has been successfully tested by using transgenic plants. Warral et al (1992) fused a modified callase gene to an Arabidopsis promoter known to be active in the tapetum. Partial or complete male sterility was observed in transgenic tobacco plants expressing the callase gene during meiosis. Thus the previous views that tapetal cells act simply to provide nutrition to the microspores need to be modified. Currently male sterility can be induced through selective destruction of tapetum by fusing promoter gene. This kind of work has got tremendous application in the production of hybrid seeds.

In CMS cotton the first symptom of aberration in tapetal function is the precocious dissolution of the callose wall around microsporocytes. We propose that the timely production, secretion or action of the callase enzyme may be essential for the initiation and completion of the meiotic division of microsporocytes. In recent years, tissue specific gene expression in pollen and tapetum have been successfully isolated and identified in number of plants (Ursin et al 1989; McCormick et al 1991; Davies et al 1992; McCormick 1993; Zaki and Dickinson 1992 and references therein). Successful creation of transgenic male sterile plants by using an RNA$^*_lase$ gene constructed under the control of a strong anther promoter, as in tobacco (Ursin et al 1989), Oenothera (Brown and Crouch 1990), tabacco and Brassica (Mariani et al 1990). Several male sterile genes have been now identified (Worral et al 1992). Currently isolation of male sterile genes is under extensive study. Recent studies on CMS bean (Abad et al 1995) indicate that the expression of at least some of these mitochondrial gene products is confirmed to some anther tissues, especially the tapetum. Similar tissue specific changes in the expression of mitochondrial genes (Conley and Hanson 1994,
Smart et al (1994) and nuclear genes encoding mitochondrial products (Johns et al 1993; Huang et al 1994) have been recently demonstrated in higher plants. Direct evidence for involvement of mitochondrial genes in anther development has been provided by Kofer et al (1991). They produced a male sterile cultivar of *Nicotiana* with common nuclear origin, but cytoplasts from different species. The observations of Kofer et al (1991) indicate that change in mt-DNA is always associated with changes in floral phenotype and differences in floral phenotype are always predictive of changes in mt-DNA. Two separate mitochondrial genes are involved in petal development, and at least one in stamen development. Thus CMS provides one of the few systems in which nuclear-mitochondrial interactions and their role in plant development can be investigated.

Further evidence for involvement of mitochondria in sterility has come from biochemical studies on male sterile anthers which revealed the disturbed activities of various mitochondrial enzymes and their multiple forms - isoenzymes. Difference in isoenzyme pattern from anther tissue of fertile and sterile wheat plants have been studied by SDS-PAGE technique (Hohler and Borner 1980). The sterile anthers of wheat lack peroxidase, esterase and leucine aminopeptidase whereas in fertile anthers they are present. A deficient cytochrome oxidase system is observed in maize (Watson et al 1977), *Sorghum* (Alam and Sandal 1969) and rice (Dai et al 1978). Loseva et al (1974) found a lower intensity of oxidative phosphorylation in mitochondria of sterile anthers and increase in ATPase which led to a lower ATP content in maize. Similarly in *Petunia*, Liu et al (1988) observed a more efficient export of ATP from mitochondria of fertile than from mitochondria of CMS plants. Few banding patterns and activities of malate and succinate dehydrogenases in maize (Ohmasa et al 1976; Palilova et al. 1977), *Wheat* (Baidulova - Babko 1983) and glutamate dehydrogenase in maize (Loseva and Mikulich 1979) are found in sterile anthers. In contrast increase in
cytochrome oxidase activity is reported in maize (Markova 1983). These studies clearly point out the functional importance of mitochondria for normal development of anther. RFLP (Restriction fragment length polymorphism) studies have revealed that male sterile genes responsible for CMS, e.g. URF 13 gene in CMS-T maize, COX-II in Beta, S-pcf in Petunia, COX-I in Sorghum, ORF-H 522 in sunflower, atp-6 in rice, and orf 138 in radish. These genes encode certain specific polypeptides which may be involved in pollen abortion. In maize CMS-T urf encodes a 13 kd protein (Leving 1990), while COX-II gene in Beta brought about transcriptional variation (Senda et al 1991). S-pcf gene in Petunia encodes 25kd protein (Nivison and Hanson 1989), COX-I in Sorghum encodes 42 kd protein (Dixon and Leaver 1982), orf-H522 in sunflower encodes 15 kd protein (Monegar et al 1994) and orf-138 gene in radish encodes 20 kd protein (Krishnasamy and Makaroff 1994). Similar variations in translation products from mutant mitochondria have also been noticed in Vicia (Boutry and Briquet 1982), Triticum (Boutry et al 1984), Nicotiana (Hakansson et al 1988), Capsicum (Manoharan et al 1993) and cotton (the present study). The functional relationship of these additional polypeptides with male sterility is not yet clearly established in most of the plants.

Our developmental and histochemical studies elucidated what we interpret as the timing of expression of CMS gene(s). In cotton, the sterility is linked to precocious dissolution of callose and collapse of meiocytes before meiosis. SDS-PAGE of anther protein from CMS and fertile anthers shows presence of a 12 kd protein in fertile anthers at sporogenous, PMC, and tetrad stage and its absence in CMS anthers at any stage. Furthermore, mitochondria isolated from CMS cotton synthesize additional polypeptides, a result which is in agreement with reports on other CMS systems. At present, it is not possible to correlate the synthesis of these additional mitochondrial
polypeptides with anther protein pattern. Further studies are very much required to understand the function of additional polypeptides synthesized by mitochondria in CMS cotton. Integrated approach of this sort are essential in other CMS systems to understand the mechanism of male sterility.