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Histology of Pollen Degeneration in Cytoplasmic Male Sterile (CMS) Cotton (*Gossypium hirsutum* L.)

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Cytoplasmic male sterile (CMS) and fertile lines of cotton are studied with special reference to the time of breakdown and fate of callose during microsporogenesis. In sterile line pollen mother cells (PMCs) abort at pre-meiotic stage or at prophase-I, through vacuolation. The deposition of callose around PMCs of fertile and CMS lines appears normal. Degeneration of callose is pre-meiotic in the sterile. The tapetum is normal.

Key Words: Cytoplasmic male sterility, Callose, Cotton, Pollen mother cells, *Gossypium hirsutum* L.

Introduction

Cytoplasmic male sterility (CMS), a maternally-inherited mitochondrial-encoded trait, is highly useful in plant breeding programmes for hybrid seed production, and is also well-known in crop plants. Although, CMS has been reported in 342 species and species crosses, the course of microsporogenesis and its breakdown stages are known only in 12% of CMS plants. Of these, in 16% the tapetum is normal, in 35% it is abnormal, and in 49% it is persistent. However, tapetal breakdown during microsporogenesis has been observed in all the cases (Kaul 1988). There is scanty information about the causes of breakdown though several morpho-physiological deviations during microsporogenesis are regarded to produce male sterility (Laser & Lersten 1972, Shivanna & Johri 1985, Kaul 1988). It is now increasingly clear that such abnormalities from the normal course of development during microsporogenesis are controlled by male sterile (MS) gene(s). The studies on histological and cytological aspects of CMS of different species have revealed that failure of microsporogenesis may occur at any stage during its development and probably more than one mechanism is involved (Manoharan 1993).

In upland cotton (*Gossypium hirsutum* L.) stable and dependable CMS lines are available. There is paucity of information concerning histological aspects of CMS in cotton. Therefore, in the present study an attempt has been made to explore the histological sequence of events of microsporogeneis which lead to sterility. The crucial role and aberrant behaviour of callose has been emphasized.

Materials and Methods

The seeds of CMS "Deshams 16" and maintainer lines of cotton were obtained from the Central Cotton Research Institute, Nagpur, India. They were sown in pots. For histological studies, dif-

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ferent stages of flower buds were fixed in Carnoy's fixative, processed and embedded in paraffin and microtomed at 6 μm". The paraffin ribbons were mounted on the slides by using 0.5% gelatin adhesive. The sections were cleared and stained in mercuric bromophenol blue (Mazia et al. 1953). For callose localization, sections were stained with 0.001% decolorized aniline blue solution in 0.1M phosphate buffer at *p*H 8.2 for 10 to 20 min (Gahan 1984), and observed under Fluorescence microscope using appropriate filter.

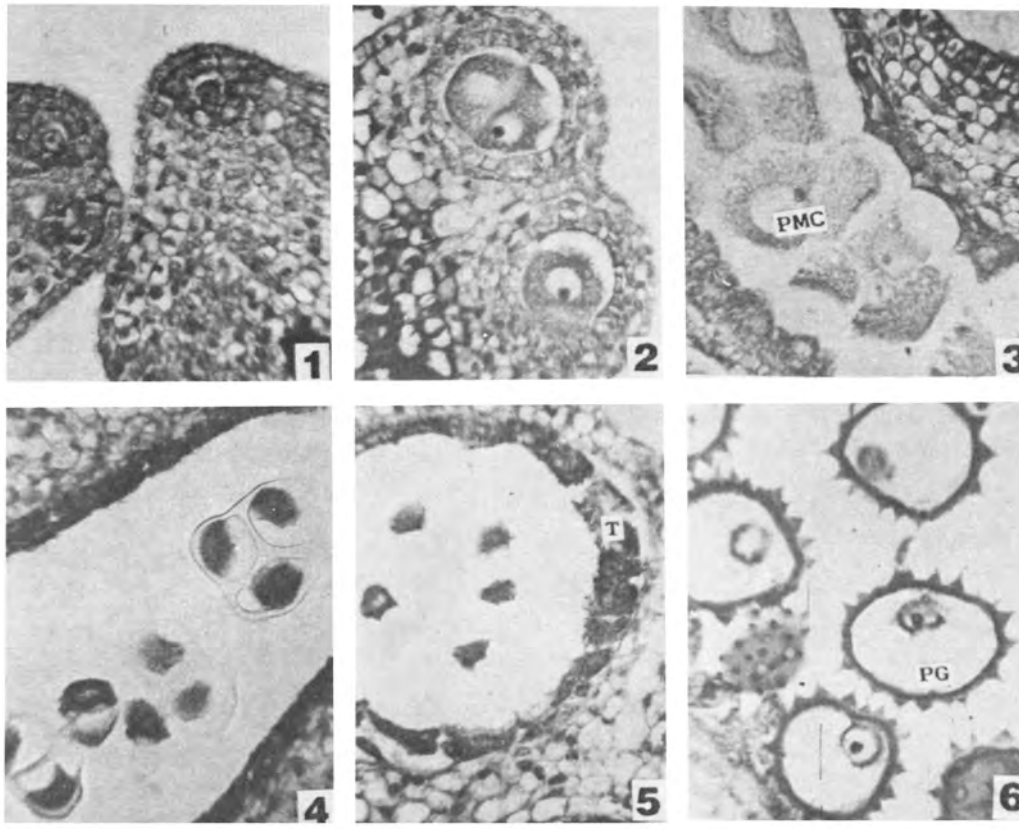
Results

The study of microsporogenesis in CMS and its fertile counterpart revealed that the pollen development initially follows same course of pattern as described for many species. The archesporial cells at hypodermal region divide to give rise to an outer layer of primary parietal cells and an inner one of primary sporogenous cells (figures 1,7). The primary parietal cells undergo further divisions resulting in the formation of an endothecium, a single middle layer, and a uniseriate tapetum, which surrounds a layer of sporogenous cells (figures 2,8). The primary sporogenous cells divide repeatedly to give rise to PMCs (figures 3,9). In the fertile line PMCs undergo meiosis and produce tetrads of tetrahedral type (figure 4), at times isobilateral type. The tapetum begins to disorganize only after microspores are released from the tetrad (figures 5,6). In the sterile line, however, PMCs at pre-meiotic stage either abort or proceed only up to prophase-I. The locule appears more or less crescent-shaped in cross section. The tapetum is secretory and behaves normally during microsporogenesis.

Fluorescence microscopy of callose revealed some striking differences. In fertile line, as expected, deposition of callose around PMCs and its subsequent degeneration after tetrad formation releasing the microspores, is normal (figures 13,15). In sterile line, however, the callose gets degraded immediately (figure 14) due to precocious activity of callose (figures 16-18). Since PMCs begin to disintegrate shortly after the deposition of callose and meiosis does not proceed further. It is difficult to identify the subsequent developmental stages of abortion with precision and the following stages may be regarded as the initial effects: Vacuolation is the first detectable variation in PMCs (figures 9, 10). The vacuoles enlarge and coalesce with each other resulting in an empty locule (figure 11), and sterility (figure 12). The tapetum and other tissues of the anther, on the other hand, maintain a level of cellular integrity even after the degeneration of PMCs (figures 11, 12).

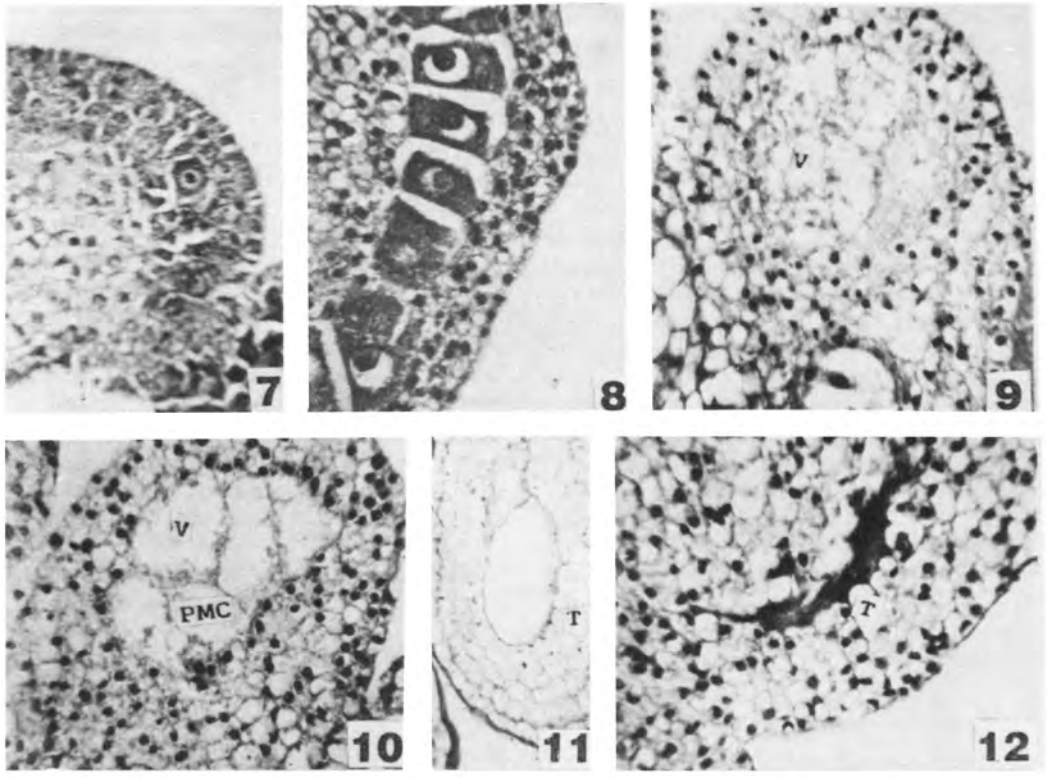
Discussion

It is well-established that the formation of pollen grains and development of the anther follow a well-defined pattern of differentiation, involving closely co-ordinated changes in the two principal cell lines: (a) the tapetum and the meiocytes, and (b) in the circumfacent anther wall tissues. Any deviation in differentiation, development and function could easily upset the balance resulting in sterility (Bhandari 1984, Shivanna & Johri 1985). This deviation in CMS reportedly occurs in the genome of mitochondria (Colhoun & Steer 1981, Bino 1985, Kaul 1988, Hanson 1991, Manoharan 1993). 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 & Meyer 1979). Comparative analysis of anther proteins from normal and CMS lines of *Capsicum* revealed the absence of 20KD protein during PMCs and subsequent stages of pollen degeneration in CMS line (Manoharan et al. 1993). Moreover, during microsporogenesis PMCs at pre-meiotic stages are enclosed by an extra-cellular matrix, namely, callose (β-1,3 polyglucan). After the completion of meiosis, callose is degraded by the activity of the enzyme callase (β-1,3-polyglucanase), leading to the release of microspores from the tetrad.



Figures 1-6 Developmental stages of fertile anther of cotton (*PG*, pollen grain; *PMC*, pollen mother cells; *T*, tapetum; *V*, vacuole). **1-2**, showing early and late sporogenous cells, respectively; **3**, pollen mother cells; **4**, tetrad; **5-6** microspores and pollen grains, respectively

Nevertheless, a number of observations strongly suggest a multitude of essential functions of callose deposits and degradation products during microsporogenesis (Malik et al. 1985). In both fertile and sterile lines of cotton, the deposition of callose around PMCs appears normal. In sterile line, pre-mature dissolution of callose by the enzyme leads to sterility. Similar observations of precocious or delayed activity of the enzyme callase have been reported in Petunia (Izhar & Frankel 1971), *Sorghum* (Overman & Warmake 1972), *Phaseolus* (Pritchard & Hutton 1972), Pisum (Gottschalk & Kaul 1974), *Capsicum* (Horner & Rogers 1974), *Allium* (Nanda & Gupta 1974) and *Lolium* (Hayward & Mantriratna 1979). Apparently, in all these taxa male sterility is caused due to faulty timing of enzyme digestion of callose wall. Callose production and accumulation prior to meiosis, and its deposition around PMCs before prophase-I, are pre-requisites for the onset of a synchronous and normal meiotic division in higher plants. Likewise, callose degradation is also essential for microspores release from the tetrad, but callose activity during early meiotic stages is deterimental. It is known that the enzyme callase is synthesized in tapetum (Pacini et al. 1985) controlled by the MS genes in

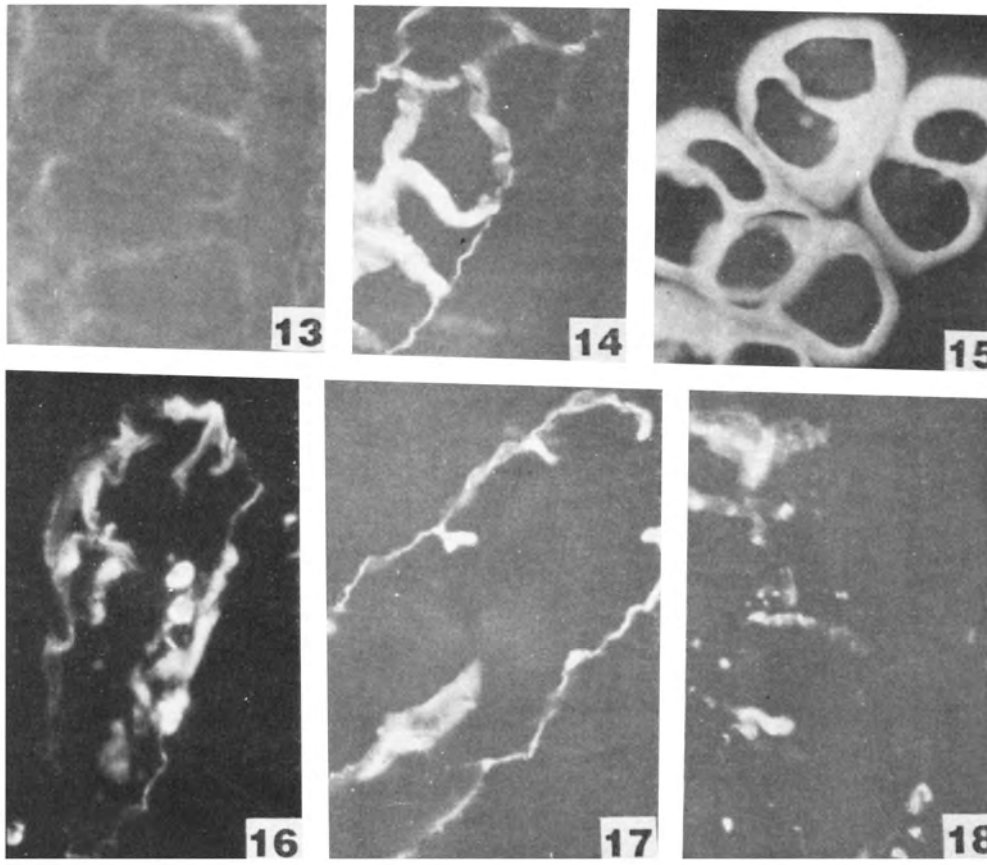


Figures 7-12 Developmental stages of sterile anther of cotton. **7-8** showing early and late sporogenous tissue, respectively; **9-10** high vacuolation in PMCs; **11** empty anther loculus; **12** complete degeneration of PMCs, and normal tapetum

the anther locule. The callose around the PMCs is digested due to precocious synthesis of callase. Consequently, the PMCs do not undergo meiosis and degenerate in cotton.

The development of sporogenous tissue to PMCs is similar in both fertile and sterile lines. However, formation of vacuoles in PMCs and their subsequent coalescence disturbs microsporogenesis in sterile line. Disorganization of tapetum and coalescence of PMCs during premeiotic stages in upland cotton was also recorded by Murthi and Weaver (1974). On the contrary, Thombre and Mehetre (1979) reported the failure of formation of PMCs and persisent tapetum in the male sterile cotton.

The anther tapetum plays a very important role in pollen development. Because of its strategic position, this tissue assumes a vital nutritive role especially during and after meiosis. There have been several reports that the tapetal cells produce several metabolites necessary for the loculus synthetic process in developing anther (Rudramuniyappa & Manure 1993). The timing of release of metabolites from the tapetum and the correlated development of microspores is very well synchronized (Pacini et al. 1985). This process of inter tissue relationship disruption almost invariably leads to pollen degeneration (Frankel & Galun 1977, Bhandari 1984). Indirect and circumstantial evidences implicate abnormal



Figures 13-18 Deposition of callose during microsporogenesis in fertile and sterile lines of cotton. **13, 14** early PMCs of fertile and PMC at prophase-I of sterile anther; **15** normal deposition of callose around the tetrads; **16-18** precocious disssolution of callose during PMCs stage in sterile anther

tapetal behaviour which triggers male sterility in higher plants (Shivanna & Johri 1985, Kaul 1988). In cotton, the tapetum maintains cellular integrity (morphologically) even after the degeneration of PMCs. The involvement of tapetum in causing male sterility in cotton can not be ruled out, in spite of the contradicting observations of Murthi and Weaver (1974) and Thombre and Mehetre (1979). However, in some taxa, the abortion of PMCs is not releated to the abnormal behaviour of tapetum, but principally to the aberrant behaviour of PMCs themselves as revealed through EM studies in *Glycine max* (Albertsen & Palmer 1979). In sunflower (Laveau et al. 1989) PMCs degenerate due to enlargement of tapetum cells. Aberrant behaviour of tapetum of various types has been recorded in several CMS lines (Bhandari 1984, Shivanna & Johri 1985, Bino 1985, Patil et al. 1993, Manoharan 1993). It may be concluded from the various studies made so far that the stage(s) at which microsporogenesis breaksdown, and the biochemical process involved in causing male sterility are highly variable in different systems.

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