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Pollen Abortion Mechanism in Genetic Male Sterile Cotton (*Gossypium hirsutum*) L.

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ABSTRACT. *The ontogenic studies of anthers in genetic male sterile (GMS) cotton against fertile cotton revealed that the development of anthers in the male sterile (MS) line appears normal until the tetrad formation. The dissolution of callose wall around tetrads of both resulted in the release of free microspores. However, considerable changes in further development of microspores in GMS line compared to the normal counterpart was noticed thereafter. In GMS line, male sterility is perhaps due to unusual vacuolation of microspores associated with convolution and disintegration of nucleus and shrinkage of microspore cytoplasm, and also due to vacuolation of tapetum at very low frequency.*

INTRODUCTION

Male sterility has gained more interest because of its potential practical value in producing commercial hybrid seeds (Rogers and Edwards, 1952; Stephens, 1937). Inspite of its importance in plant breeding, the causal mechanisms of cytoplasmic (Edwardson, 1970; Laser and Lersten, 1972) and genetic (Jain, 1959; Gottschak and Kaul, 1974) male sterility in plants are not clearly understood. A considerable histological basis of male sterility has been reported in different crops, such as, wheat (Chauhan and Singh, 1966), sugarbeet (Artschwager, 1947) and soybean (Dubey and Singh, 1965). Although several cytoplasmic and genetic male sterile genotypes of *G. hirsutum* cotton ranging from partial sterility to complete sterility have been reviewed (Bowman *et al.*, 1978; Murthi and Weaver, 1974), very few reports are available on the ontogenic aspects of cotton. The present research on pollen abortion mechanism in GMS cotton was carried out to characterize the nature of irregularities during microsporogenesis and pollen development.

MATERIALS AND METHODS

Flower buds at various stages of development were collected from both GMS line (Gregg MS) and fertile line of cotton grown in cotton fields during summer 1992-93. The buds were fixed in Cornoys A (3 absolute alcohol:1 acetic acid) solution. The anthers were dehydrated with ethanol and tertiary butyl alcohol series and embedded in paraffin wax. Standard microtoming technique was followed to obtain cross sections of 8-12 μ thickness which were stained with bromophenol blue.

RESULTS AND DISCUSSION

The comparative observations made in GMS and fertile anthers revealed that the process of microsporogenesis in male sterile anther paralleled that in the fertile anther until shortly after meiosis. Pollen development in the sterile anther began to diverge from fertile anther just after meiosis, as the tetrads of microspores were released from their callose walls.

The young anthers in both sterile and fertile line showed multicellular archesporium in each lobe, which divided periclinally to produce an outer layer of primary parietal cells and an inner layer of sporogenous cells. The further anticlinal and periclinal divisions of primary parietal cells differentiated in an outer endothecium, a middle wall layer and a layer of tapetum (Figure 1a). With few mitotic divisions, the sporogenous cells produced a large number of microspore mother cells (Figure 1b), which resulted in the formation of microspore dyads and tetrads by normal process of meiosis (Figure 1c). The dissolution of callose wall around the tetrads resulted in the formation of free microspores (Figure 1d). However, considerable changes observed in subsequent developmental stages of microspores in GMS line compared to normal counterpart was noticed thereafter.

Pollen development in male fertile anther

After the release of microspores from the tetrads, microspores enlarged considerably (Figure 2a) and the exine pattern typical to cotton was laid down around them during further development in the fertile anther (Figure 2b).

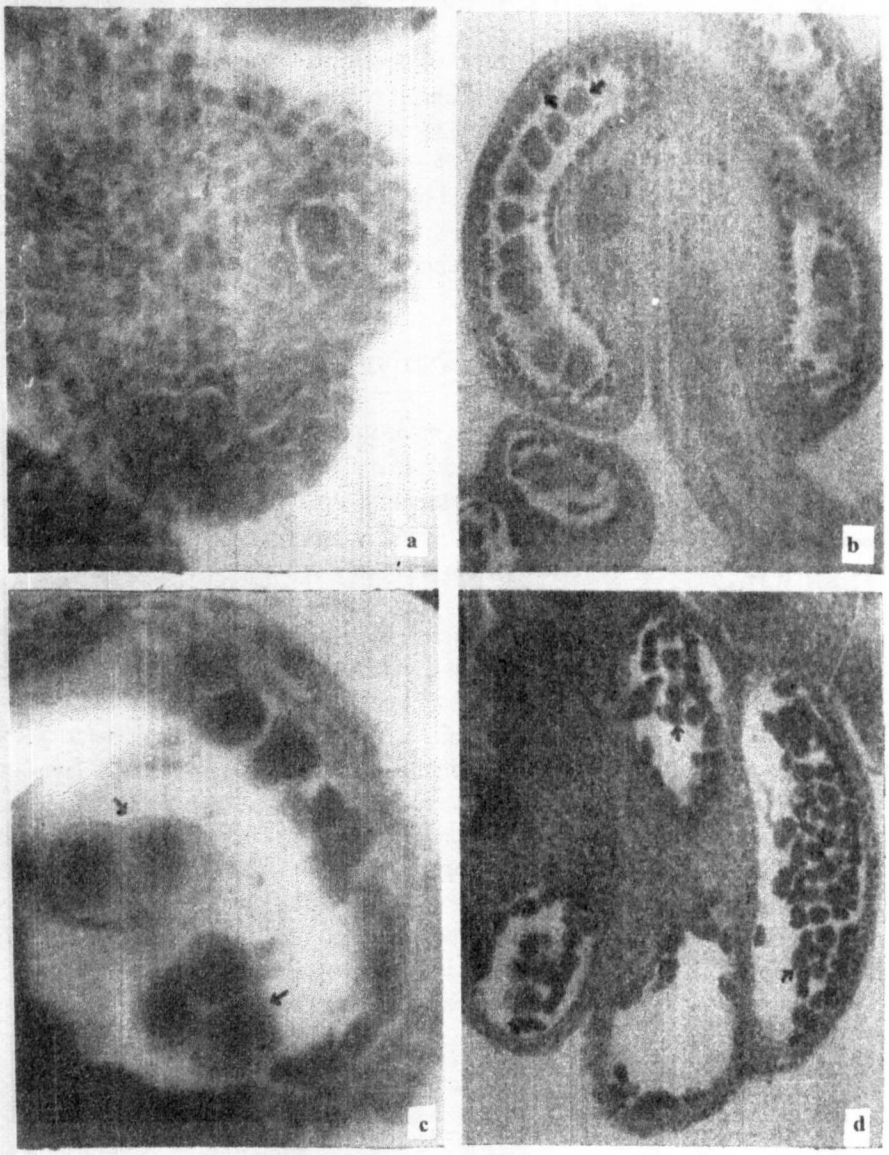

Figure 1. Formation of free microspores in the anthers of sterile lines of cotton. (Only formation of free microspores in GMS line is depicted and not of fertile line since the process is same in both the lines until microspore release) a) T.S. of sterile anther showing 3 wall layers around PMC's. b) L.S. of fertile anther showing PMC's. c) T.S. of sterile anther showing tetrads (di and trihedral) with intact tapetum. d) T.S. of sterile anther showing free microspores with disintegrating tapetum.

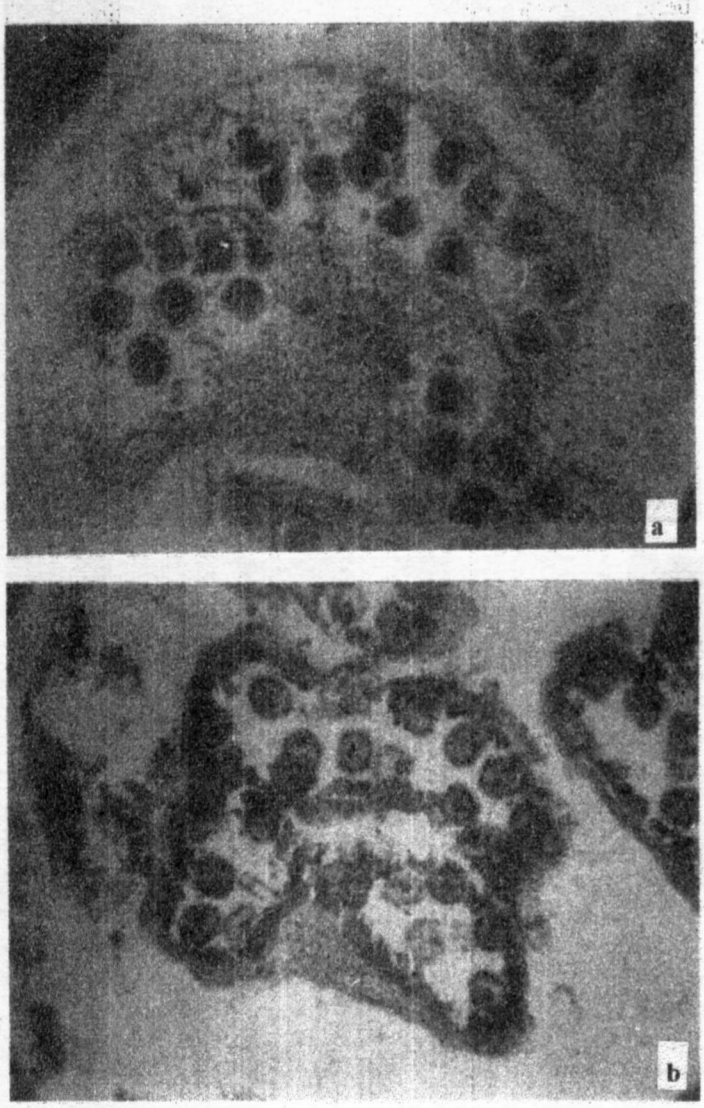

Figure 2. Pollen development in male fertile anther of cotton.
a) T.S. of anther showing a darkly stained and enlarged microspores.
b) T.S. of anther showing normal pollen grains.

Pollen abortion in male sterile anther

In GMS cotton, although the microspores enlarged initially, subsequently they became considerably shrivelled due to the formation of vacuoles, shrinkage of cytoplasm and degeneration of chromatin material. Further increase in vacuolation resulted in the formation of deformed pollen grains (Figure 3a and 3b). Hence, the formation of exine pattern typical to cotton was hindered in the pollen grains of GMS line (Childers, 1952; Orel, 1968; Regan and Moffat, 1990). The deformed pollen grains ultimately disintegrated. Thus, the anther sacs during anthesis appeared either empty (Figure 3c and 3d) or with thick black mass at the centre (Figure 3e); and at a still later stage, the anther cavity was occupied by a mass of tissue, leaving little (Figure 3f) or no space (Figure 3g). Such abnormal changes after the formation of microspores resulted in disintegration of pollen grains, bringing about male sterility.

The abnormality associated with the further development of released microspores was most likely due to nutrient deficiencies. The developing microspores require large amounts of nutrients for their growth and differentiation (Cooper, 1952). Either the supply or inability of developing microspores to absorb the nutrients might be the reason for the pollen abortion in this paricular type of male sterile line. However, the functional basis of male sterility may be due to complete absence or scarce activity of some biochemical substrates/precursors/enzymes (Duvick, 1965; Regan and Maffat, 1990; Saini and Davis, 1969; Van de Meer *et al.*, 1992).

The tapetal behaviour in both GMS and fertile anther was found to be normal during the course of anther development. The tapetum differentiated simultaneously with sporogenous tissue, which persisted during meiosis till the formation of microspores, but, began to disintegrate in GMS line (Figure 1d). Degeneration of microspores during or after the pollen wall differentiation without involving the tapetal abnormality was reported in wheat (Fukaswa, 1956) and rice (Chu *et al.*, 1972). However, at an extremely low frequency, apart from the abnormal behaviour of released microspores, tapetal cells also showed enlargement accompanied by a high degree of vacuolation (Figure 3a) in some anther locules (Kaul and Singh, 1966 in barley; Khadi *et al.*, 1994 in cotton and Artschwager, 1947 in sugarbeet). The tapetum plays a vital role in normal development of pollen grains (Maheshwari, 1950). Although what causes the enlargement of tapetal cells is not clearly known, the speculations with regard to accumulation of excess food material transported from other sporophytic tissues, and the inability of the tapetum to transfer food materials beyond it cannot be ruled

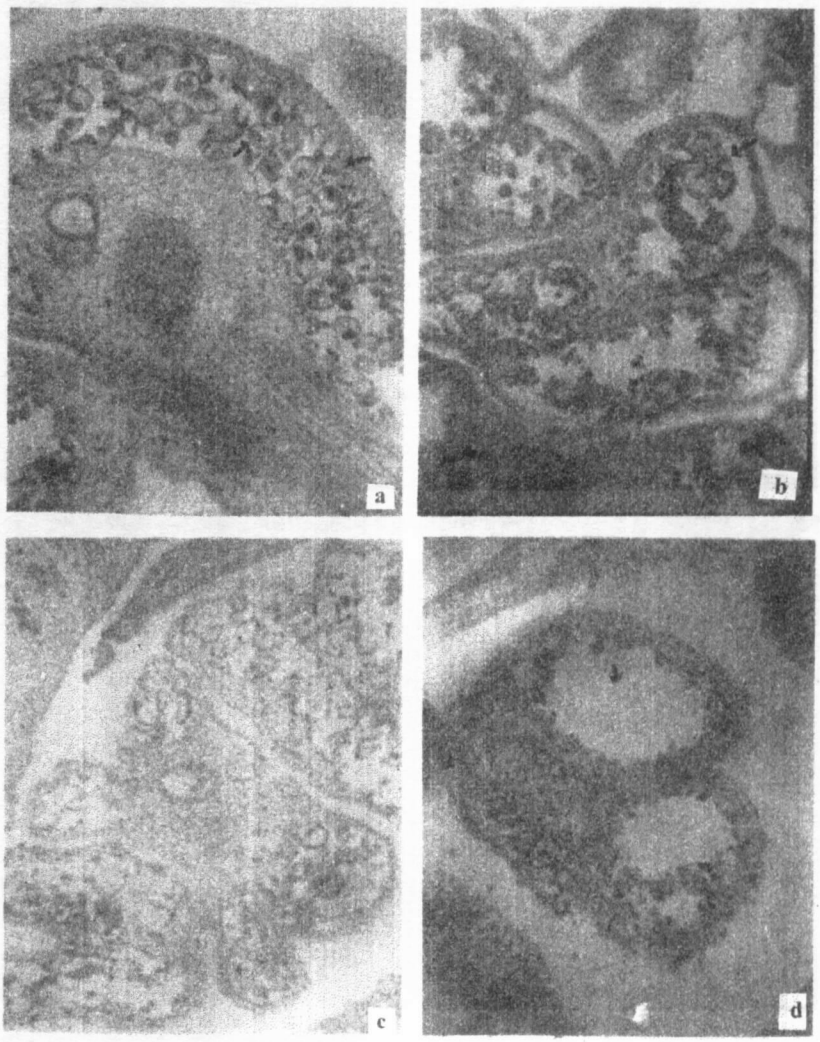

Figure 3. Pollen abortion in male sterile anther of cotton.
(a) L.S. of anther showing enlarged microspores due to vacuolation and hypertrophoid tapetum. (b) T.S. of anther showing shriveled pollen-grains and degenerated tapetum. (c) T.S. of anther showing pollen wall disintegration. (d)T.S. of anther showing almost empty anther sac.

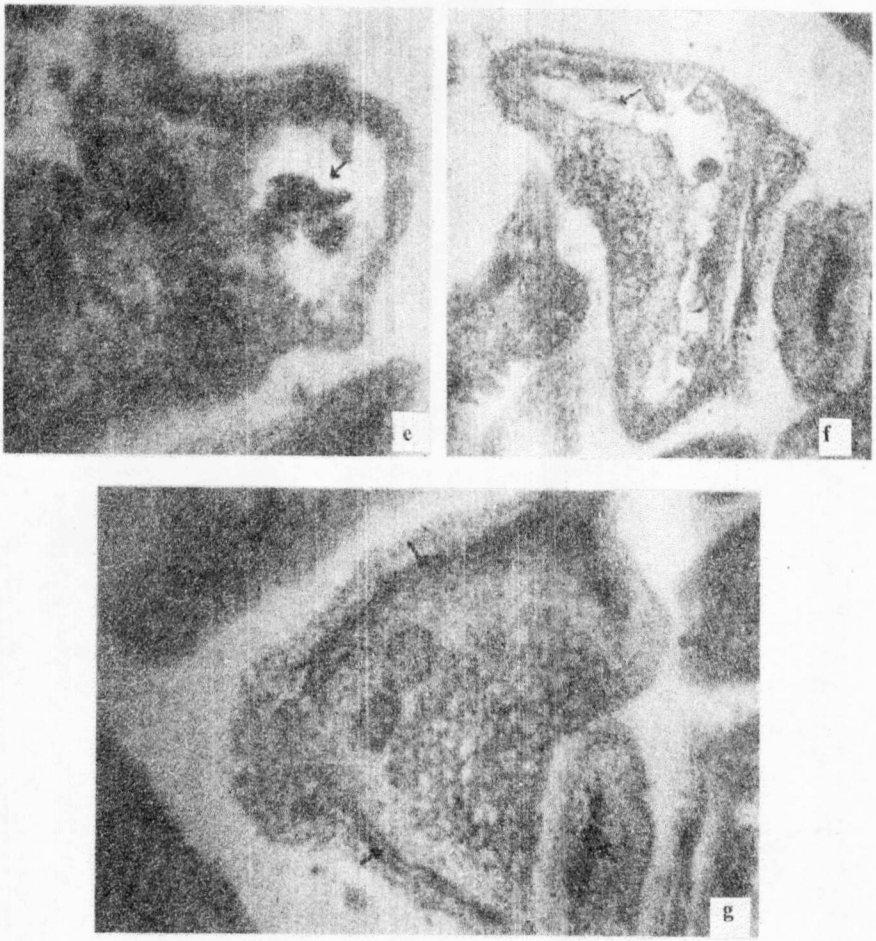

Figure 3. Pollen abortion in male sterile anther of cotton (continued).
(e) T.S. of anther showing thick black mass of tissue at the center of anther sac. (f) L.S. of anther cavity having very little space. (g) L.S. of anther showing an anther cavity being occupied by a mass of tissue leaving no space.

out. However, the vacuolation of tapetal cells may be reasonably attributed to the inefficiency of tapetum to absorb incoming nutrients, since vacuolation of tapetum in some anther locules of GMS cotton strain was not clearly detectable in all the anthers.

CONCLUSIONS

From the present investigation it can be stated that in GMS cotton, male sterility is perhaps due to the unusual vacuolation of microspores associated with convolution and disappearance of nucleus. The shrinkage of microspore cytoplasm hinders the development of pollen grain, and also, the vacuolation of tapetum at a lower frequency leading to male sterility in GMS cotton.

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