

## Histological aspects of microsporogenesis in genetic male sterile and fertile DS-5 lines of *G. arboreum* L. cotton

Although few desi cotton hybrids, namely DDH-2, G. cot. DH-7, DH-9 and MDCH-201 have been released for cultivation in the recent past, area under these hybrids is not significant due to uneconomic hybrid seed production. The cost of hybrid seed is 15-20 times higher than the pure varieties. Thus the conventional hybrids are beyond the reach of marginal and poor farmers. Use of genetic male sterility

can considerably reduce the cost of hybrid seed production by elimination of manual labour cost required for emasculation. Petaloidy nature has been observed in *G. arboreum* L.<sup>1</sup>, in which anthers are transformed to petal-like leafy structures. This has not been utilized for hybrid seed production due to its unstable behaviour. Recently a new GMS line namely DS-5 was identified wherein sterility is

governed by a single recessive gene (*ams1*)<sup>2</sup>. The present investigation was planned to know whether the sterility in this line is due to premeiotic or post-meiotic abnormality during microsporogenesis through the comparative histological studies between sterile and fertile anthers. Such information helps to know stability of male sterile source, for its further use in hybrid seed production.

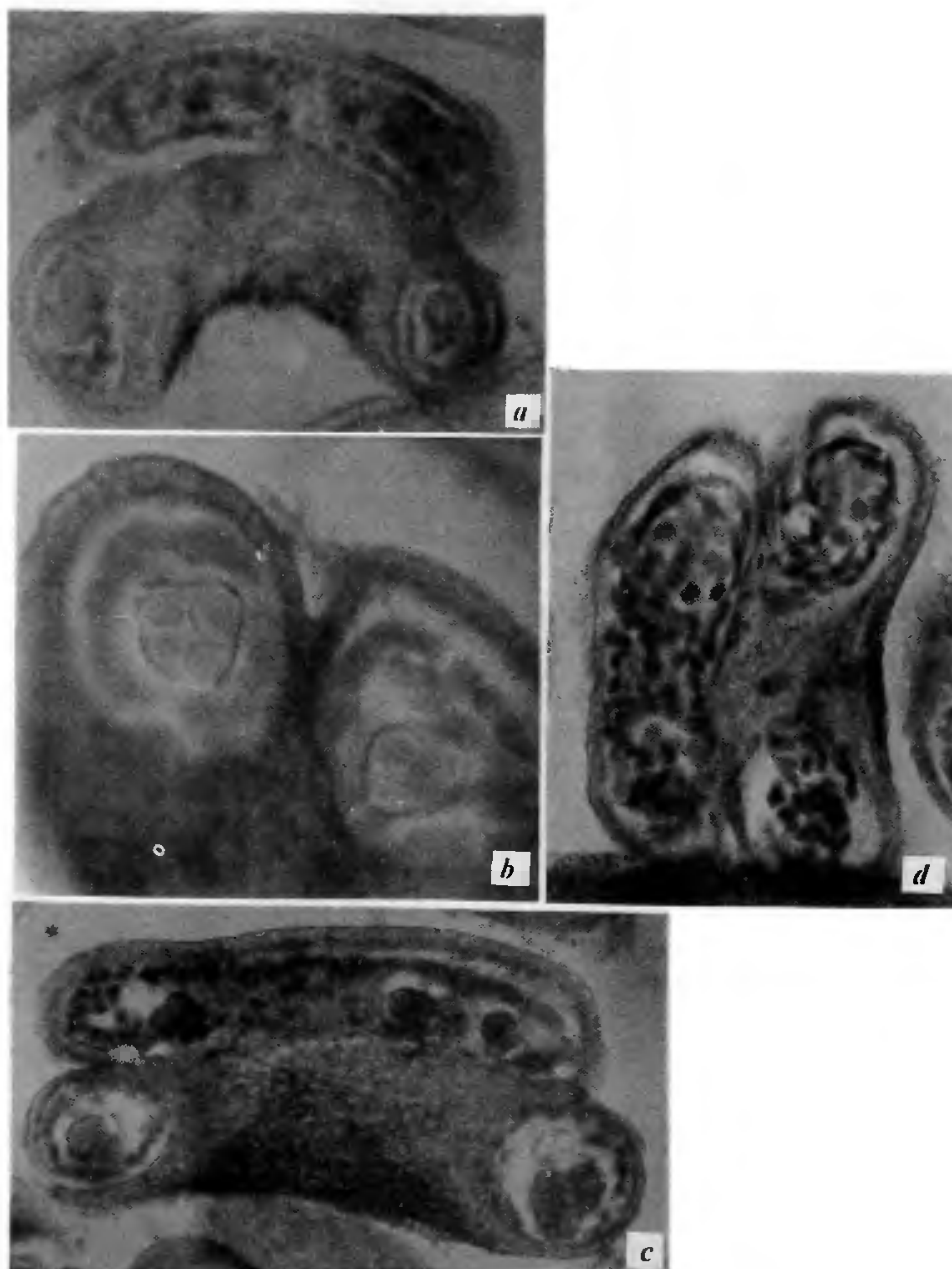


Figure 1a-d. Cross sections of anther showing differentiation of epidermis, middle wall layers, tapetum and pollen mother cells in DS-5 male-sterile (a), and fertile cotton genotype (b). Intact trihedral and tetrahedral tetrads in male sterile anther locule with disintegrating tapetum (8x × 40x); c, tri- and tetrahedral tetrads in male fertile anther; d, Released microspores with disintegrating tapetum in male fertile anther.

Male sterile and fertile flower buds of DS-5 line were fixed in Carnoy's A solution, and the buds were allowed to remain in fixative for 48 h. The detailed histological studies were carried out by adopting standard methods of microtechnique<sup>3,4</sup>. The tissues were stained using Heidenhain's iron haematoxylin (0.5%), and observed under a light microscope.

The study of microsporogenesis in anthers of GMS and its fertile counter line indicated the same pattern until the release of microspores from the tetrads. The archesporial cells in both GMS and fertile lines were differentiated in each anther lobe to give rise to outer layer of primary parietal cells and inner layer of primary sporogenous cells. The primary parietal cells further, with anticlinal and

periclinal divisions, differentiated into endothecium, a middle wall layer, and sporogenous cells in both GMS and fertile anthers. The sporogenous cells with further meiotic divisions resulted in formation of pollen mother cells in male sterile anther (Figure 1 *a*). The pollen mother cells through normal meiosis both in sterile and fertile anthers resulted in formation of microspore dyads and tetrads (Figure 1 *b, c*). The tapetum began to disintegrate soon after the release of microspores from tetrads, although it appeared at premeiotic stage and continued to persist during meiosis (Figure 1 *d*). After release of the microspores, certain changes were observed during further development of the microspores into pollen grains in GMS anthers.

The microspores after release from the tetrads, enlarged considerably with dense cytoplasm taking dark stain (Figure 2 *a-c*). Each microspore later on developed the usual intine and exine wall, a characteristic spiny serrated margin leading to formation of matured pollen grains. The shedding of matured pollen grains occurred due to breakdown of outer endothecium layer (Figure 2 *d*). Each pollen grain at this stage had prominent nucleus either in center or little away from the center.

The process of degeneration of microspores started after their release from the tetrads. Each microspore enlarged in the initial stage and during further development of microspores resulted in shrivelling and shrinkage of microspore

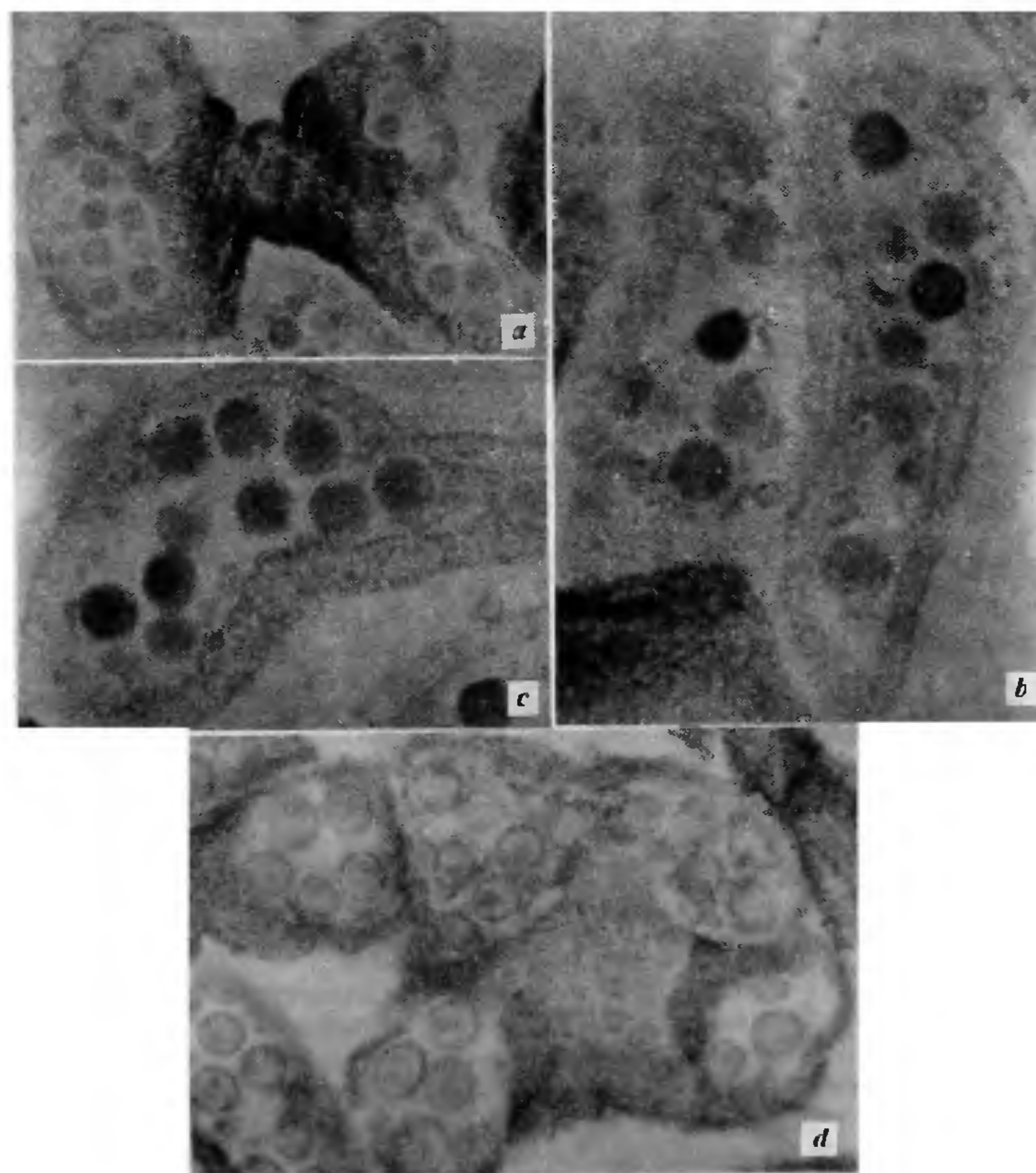


Figure 2 *a-d*. Pollen development in DS-5 male fertile cotton: (*a*, 8x × 10x) and (*b*, 10x × 10x): Enlarged microspores with dense cytoplasm; *c*, Enlarged microspores showing the differentiation of intine and exine. Note the differentiation of endothelial thickenings; *d*, Fully developed pollen grains with prominent nucleus and spiny exine pattern.

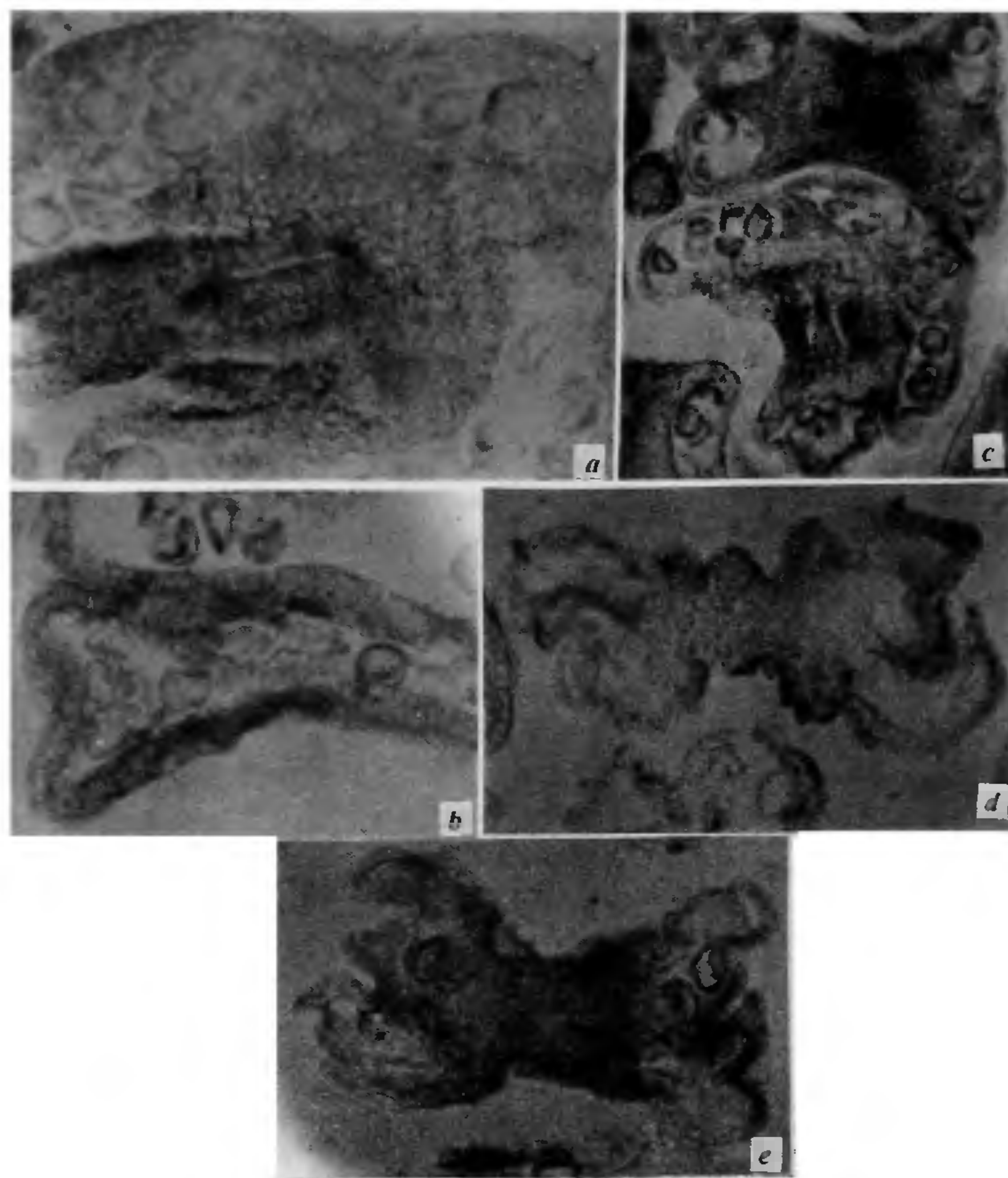


Figure 3a-e. Pollen abortion in DS-5 male sterile line. *a*, Shrivelling of microspores associated with shrinkage of cytoplasm leading to abnormal shape. *b*, Shrivelled microspores with slight serrated margin. *c*, Abnormal-shaped microspores depicting the formation of slight serrated margin. *d-e*, Anther locules showing abnormal shape enclosing deformed late microspores.

cytoplasm (Figure 3a). Similar observations were made previously in male sterile anthers of cotton<sup>5-8</sup>. The deformed microspores later developed a slight serrated margin with incomplete development of pollen wall (Figure 3b and c). The anther locules showed abnormal shape due to appression of sac walls towards the center, leading to complete disorganization (Figure 3d and e).

The development of microspores requires large amount of nutrients for growth and differentiation<sup>9,10</sup>. Lack of supply or inability of developing microspores to absorb the nutrients might be responsible for the pollen abortion in this particular GMS line. However, the functional basis of abnormal behaviour of microspore cannot be studied with the

histological observations. Since the male sterility in DS-5 line is due to post-meiotic abnormality, care should be taken for utilizing this line for commercial hybrid seed production.

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