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Development of Male Gametophyte in Fertile and C.M.S. Line of *Nicotiana tabacum*

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A comparative study of the development of male gametophyte in fertile and C.M.S line of *Nicotiana tabacum* was made. The development of anther in the fertile line was perfectly normal whereas in the C.M.S line the four anther lobes were *ab initio* unequal; the anther tapetum was monomorphic; endothecium was devoid of fibrous thickenings and there was early degeneration of tapetal cells which probably was the main cause of pollen sterility.

Key Words - Anther Endothecium Fibrous Lobe Sterility Tapetum

Rhoades (1930) reported cytoplasmic male sterility in corn and explained its use in the production of hybrid seeds. Cytoplasmic male sterile lines (C.M.S.) the laborious emasculation process can safely be avoided. Clayton (1950), Burk (1960) and Ramavarma *et al.* (1978) developed C.M.S. lines of of *Nicotiana* through interspecific hybridization and studied the associated floral anomalies. Chaplin (1969) and Sastry *et al.* (1982) through repeated back crosses from the male sterile line PDMS-1 of *N. tabacum* L. (var. Hicks) incorporated cytoplasmic male sterility to 8 devergent, elite flue - cured tobacco varieties (including the variety - C.T.R.I-special). Sastry *et al.* (1982) made a comparative study of fertile and sterile lines for quality characters and yield components. But for high nicotine content and low reducing sugars, the fertile and sterile lines did not show any difference. In this study the sequential events of anther development and microsporogenesis in both fertile and sterile lines have been studied.

MATERIALS & METHODS The flower buds at various stages of development were collected from the C.T.R.I. experimental farm at Katheru near Rajahmundry and fixed in FAA. Routine methods of microtechnique were followed (Johansen, 1940). Sections were cut at thickness ranging from 6 - 9 μ and stained with Delafield's haematoxylin.

OBSERVATIONS In the fertile line of C.T.R.I - Special, the developmental sequence in the anther wall and microsporogenesis occur normally. The anther is tetrasporangiate with Basic type of wall development (Figs. 2, 8 - 11). The secretory tapetum is uniseriate and dimorphic with uni- or bi-nucleate cells. The endothecium becomes multilayered and develops fibrous thickenings at dehiscence (Fig. 12). The sporogenous tissue is 2-layered and crescent shaped. Simultaneous cytokinesis in pollen mother cells results in tetrahedral, decussate and isobilateral pollen tetrads (Figs. 13 - 15). The pollen grains are globose, both tri- and tetra-aperturate with smooth exine and shed at the 3-celled stage (Fig. 16).

In the cytoplasmic male sterile line beside certain floral anomalies, several developmental abnormalities in the male gametophyte occur. The five stamens bear petaloid extensions on either side of the filaments and the connective extends beyond the anther lobes and develops a knob giving the appearance of style and stigma, a phenomenon called stigmatoidy (Fig. 1).

The anther lobes are quite unequal even from the 4-lobbed stage (Fig.3). The development of anther wall although follows the basic type, its different entities show anomalies in their structure and behaviour. The tapetum is monomorphic with 1- to 4-nucleate cells. The endothecum is without fibrous thickenings.

Gametogenesis in the anther is associated with unusual behaviour of the tapetum as given below:

(1) Precocious degeneration of the tapetum The tapetum in some anther lobes degenerates immediately after its differentiation, followed by the degeneration of the sporogenous tissue (Figs. 4, 17).

(2) Hypertrophy of the tapetum The tapetum grows vigorously, becomes extensive, reaches unusual dimensions and sandwiches the sporogenous tissue at different stages of development: (a) Hypertrophy of the tapetal cells dur-

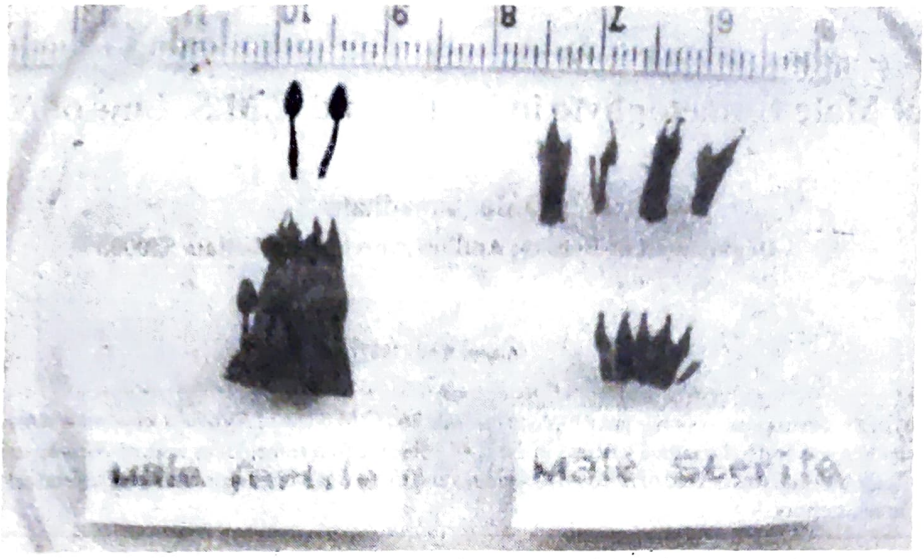
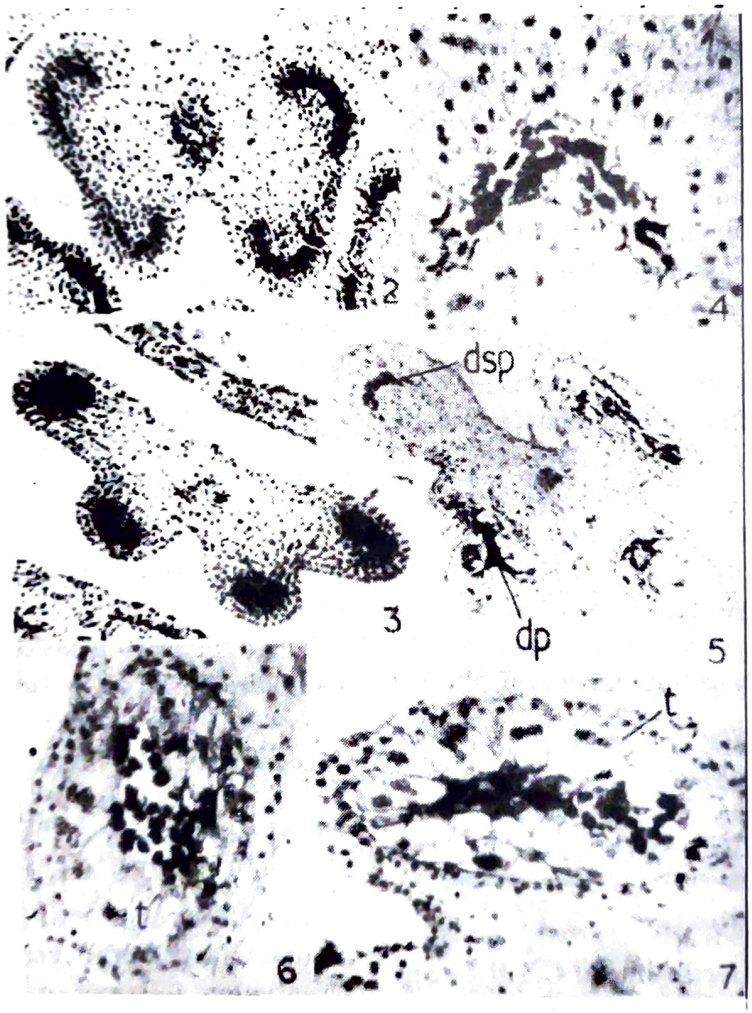
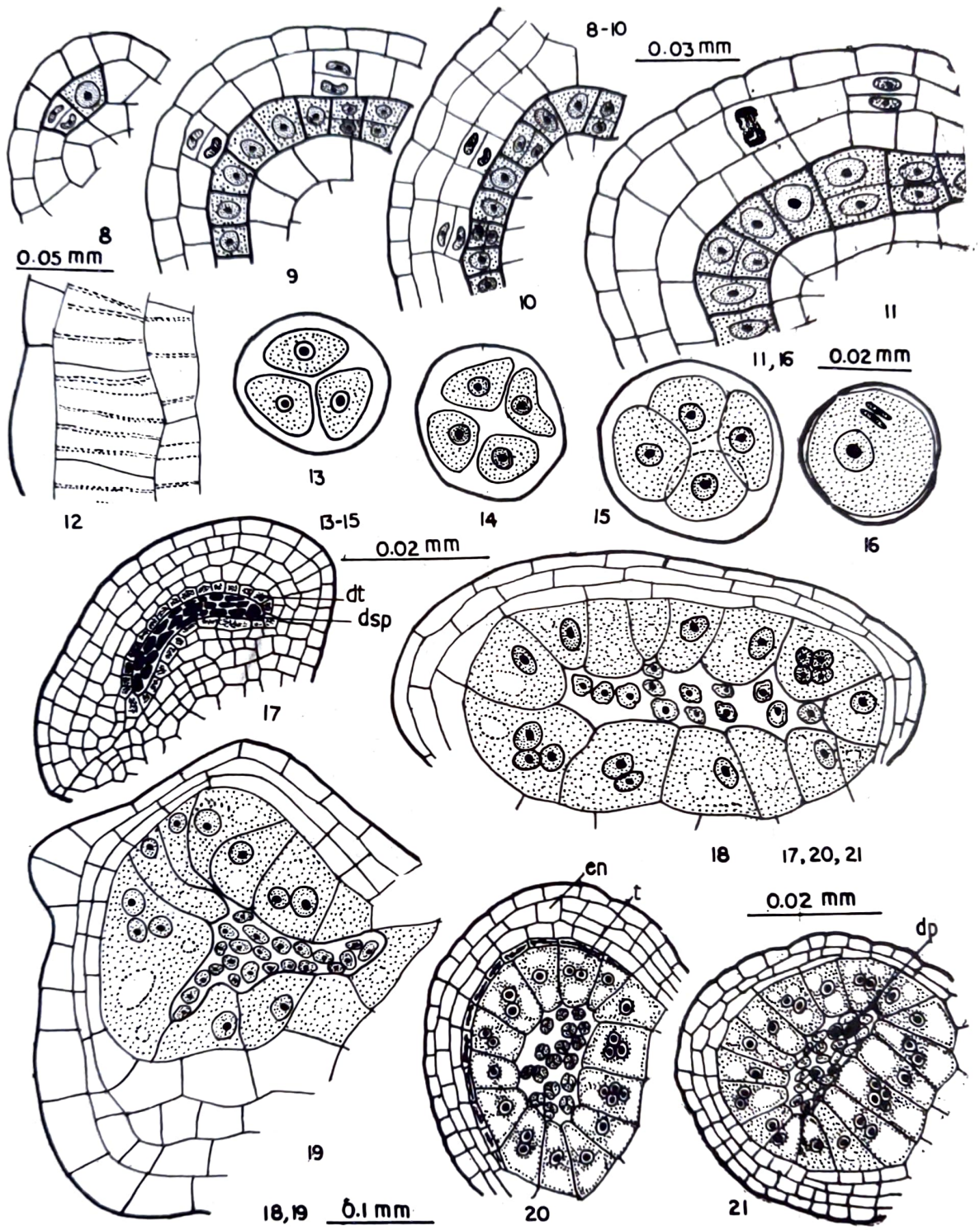


Fig. 1. Fertile line showing normal stamens and C.M.S line showing petaloidy and stigmatoidy of the stamens. (c = connective).



Figs. 2 - 7. Developmental abnormalities of the male gametophyte in C.M.S line. 2. T.S. tetrasporangiate anther showing crescent shaped sporogenous tissue in the fertile line. 3. T.S. tetrasporangiate anther showing unequal anther lobes in the C.M.S. line. 4. T.S. part of an anther lobe showing degenerating sporogenous tissue. 5. T.S. anther showing abnormalities in the four lobes. 6. T.S. part of an anther lobe showing hypertrophy of the tapetal cells during pre-meiotic stage. 7. T.S. part of anther lobe showing hypertrophy of the tapetum during post-meiotic stage. (dp - degenerating pollen grains; dsp - degenerating sporogenous tissue; t - tapetum).



Figs. 8 - 16. Stages in the microsporogenesis of fertile line. Figs. 17 - 21. Stages in the developmental abnormalities of the male gametophyte in C.M.S. line. 8 - 12. T.S. part of anther lobe showing wall development. 13 - 15. Tetrahedral, isobilateral and decussate microspore tetrads. Fig. 16. 3-celled pollen grains. 17 T.S. part of an anther lobe showing degenerating sporogenous tissue. Figs. 18, 19. T.S. part of anther lobe showing hypertrophy of the tapetal cells during premeiotic stage. 20, 21. T.S. part of anther lobe showing hypertrophy of the tapetum during postmeiotic stage (cp - crushed pollen grains; dt - degenerating tapetum; en - endothecium).

ing premeiotic stage: The tapetal encroachment starts at the sporogenous cell stage, resulting in the crushing up of the pollen mother cells (Figs. 6, 18,19). (b) Hypertrophy of the tapetum during post-meiotic stage: The radial elongation of the tapetum starts during meiosis resulting in the crushing up of either the pollen tetrads or uninucleate pollen grains (Figs. 7, 20, 21).

Further, the limited locular space of the anther is not sufficient for the differentiation and further development of the pollen tetads and pollen grains.

(3) Intratapetal syncytium The radial walls of the tapetum in some anther lobes break down, the contents coalesce and form a structure called intra-tapetal syncytium. The inner walls of the tapetal cells remain intact even at a later stage.

These abnormalities are present not only in the different anthers of the same flower but even in the four loculi of the same anther (Fig. 5).

Structural abnormalities together with functional breakdown of the tapetal tissue as illustrated by its unusual elongation, vacuolation and less dense cytoplasm and its applied pressure towards the loculus make the pollen sterile. Fertile pollen grains are never formed.

DISCUSSION A comparison of the development of the anther wall and microsporogenesis in the fertile and cytoplasmic male sterile lines of *N. tabacum* (var. C.T.R.I. - special) reveals that in the former, the developmental sequences occur normally leading to the formation of fertile pollen grains, whereas in the C.M.S. line a good number of anomalies are present. Such anomalies have been previously recorded by Chauhan & Singh (1966, 1968) in wheat and *Cucumis melo,* Overman & Warmke (1972) in *Sorghum* and Horner & Rogers (1974) in *Capsicum annuum.* Several variations in the floral parts, such as split corolla tubes, outgrowths on the corolla, apetalous flowers, petaloidy, pistillody and stigmatoidy have been recorded by earlier workers (Goodspeed, 1954; Burk, 1960; Ramavarma *et al.*, 1978). In the present investigation, the petaloid filaments and stigmatoid anthers are identical to the previous report of Goodspeed (1934).

The cause of male sterility in C.M.S. line is due to the abnormal behaviour of the tapetal cells.

In the C.M.S. line where the tapetal protoplast remains persistent, fibrous thickenings are not developed on the endothecium. This is in support of the statement made by Decossard (1969) "that some substances present within the tapetal cells inhibit the development of endothecial thickenings". Such a condition has been reported *in Allium cepa* and *Beta vulgaris, Capsicum annuum, Cucumis melo, Cucurbita maxima, Datura alba, Rananculus muricatus, Sesamum indicum* and *Triticum aestivum.* (Saini & Davis 1969 Chauhan, 1979).

The role of tapetum as a nutritive tissue during microsporogenesis has been stressed by Vasil (1967) and Eichlin (1971). But Heslop-Harrison (1972) opined that the abnormal development and functioning of the tapetum could easily upset the balance between the tapetum and the sporogenous tissue resulting in the abortion of the latter. The relationship between the abnormal behaviour of the tapetum and disorganisation of the sporogenous tissue in the C.M.S lines of *Sorghum* has been recorded by Overman & Warmke (1972), Horner & Rogers (1974) in *Capsicum*, Cheng *et al.* (1981) in *Oryza sativa.*

In all these plants including *Nicotiana*, the tapetum shows abnormalities such as formation of intratapetal syncytium, thickened inner walls, high vacuolation, hypertrophy and intrusion of the tapetum into the anther loculus. Probably, the thickened inner tapetal wall may retain the nutrients rather than transport them to the developing sporogenous tissue. I feel that precocious degeneration and hypertrophy of the tapetum are reponsible for the failure of development of fertile pollen in the C.M.S. line.

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REFERENCES

BURK L G 1960 Male-sterile flower anomalies in interspecific *Nicotiana* hybrids *Hered.* 51 27-31.

CHAPLIN J F 1969 Registration of PDMS-1 and PDMS-2 tobacco germplasm *Crop Sci* 9 601-682.

CHAUHAN S V S & S P SINGH 1966 Pollen abortion in male sterile hexaploid wheat (Norm) having *Aegilops ovata L. Cytoplasm Crop Sci.* 6 532-535.

CHAUHAN S V S & S P SINGH 1968 Studies on pollen abortion in *Cucumis melo* L. *Agra Univ J Res Sci.* 17 11-22.

CHAUHAN S V S 1979 Development of endothecium in relation to tapetal behaviour in some male sterile plants *Phytomorphology* 9 245-251.

CHENG Y K C S HUANG & K L LAI 1981 Studies on cyto plasmic genetic male sterility in cultivated rice (*Oryza sativa* L.). IV. Histological, morphological investigation on male sterility of variety Reimei having *O. rufipogon* Cytoplasm *Sci Counc Monthly Roc* 9 1095-1111.

CLAYTON E E 1950 Male sterile tobacco *J Hered* 41 171-175.

DE FOSSARD R A 1969 Development and histochemistry of endothecium in the anthers of *in vitro* grown *Chenopodium rubrum* L. *Bot. Gaz* 130 10-22.

EICHLIN P. 1971. The role of the tapetum during microsporogenesis of angiosperms. In *Pollen Development and Physiology*. Ed, J. Heslop-Harrison Butterworths London pp41-46.

GOODSPEED T H 1934 *J Genet* 29 327-353.

GOODSPEED T H 1954 The genus *Nicotiana. Chronica Botanica* Waltham U.S.A.

GOODSPEED T H & AVERY 1934 The cytogenetics of fourteen types derived from a single x-rayed sex cell of *Nicotiana tabacum. J Genet* 29 327-353.

HESLOP-HARRISON J 1972 Sexuality in angiosperms. 133-289. In Steward, F.C. (ed.). In *Plant Physiology. Physiology of development. From seeds to sexuality*. Vol 6c, ed, F C Steward, Academic Press, New York.

HORNER H T & M A ROGERS 1974 A comparative light and electron microscopic study of microsporogenesis in male fertile and cytoplasmic male sterile pepper *(Capsicum annuum) Can J Bot* 52 435-441.

JOHANSEN D A 1940 *Plant Microtechnique* McGraw-Hill New York.

OVERMAN M A & H E WARMKE 1972 Cytoplasmic male sterility in Sorghum II Tapetal behaviour in fertile and sterile anthers, *J. Hered.* 63 227-234.

RAMAVARMA K T K APPA RAO & A I NARAYAN 1978 Male sterile flower anomalies in an interspecific *Nicotiana* hybrid *Tob Res* 4 29-33.

RHOADES M M 1930 Cytoplasmic inheritance of male sterility in *Zea mays, Science* 73 340-341.

SAINI S S & G N DAVIS 1969 Male sterility in *Allium cepa* and some species hybrids *Econ Bot* 23 37-49.

SASTRY 1982 Effect of cytoplasmic male sterility from *Nicotiana undulata* on yield, yield components, chemical and physical quality characteristics of fluecured Tobacco genotypes *Tob Res* 8 173-177.

VASIL I K 1967 Physiology and cytology of other development *Biol Rev* 42 327-373.