Observations
OBSERVATIONS

HISTOLOGY

Microsporogenesis in Fertile and Cytoplasmic Male Sterile (CMS), Raphanus sativus L.

The pattern of microsporogenesis in fertile and CMS anthers of Raphanus follows same course of development up to the release of microspores from a tetrad stage. The anther is tetrasporangiate. Each sporangium at pollen mother cells consists of an outer epidermis subtended by an endothecium, one or two middle wall layers and secretory tapetum. Each of meiocytes and its tetrads is normally enclosed by a callose wall. The tapetum remains persistent even after the formation of pollen grains in the fertile line. In CMS, on the other hand, the first sign of abnormality appears in tapetum. Soon after the microspores are released from a tetrad, tapetal cells walls begin to rupture, allowing the cellular contents of the tapetum to flow together into the locule. These contents remain enclosed within a continuous innertapetal wall layer which forms an intratapetal syncytium. Subsequently, intratapetal syncytium of tapetum and microspores start degenerating simultaneously in the sterile line. Rarely complete hypertrophy of tapetum is observed. Even after the collapse of the sporangial locule, epidermis, endothecium, middle wall layers remain persistent.

Microsporogenesis in Fertile and Cytoplasmic Male Sterile (CMS), Pennisetum typhoides Stapt-et-llubb.

The anther is tetrasporangiate, development of anther wall follows the monocotyledonous pattern and each sporangium has four wall layers. The outermost is epidermis subtended by endothecium, middle wall layer and secretory tapetum. Following meiosis, partition walls in tetrad formation are formed perpendicular to the tapetum, and each tetrad is enclosed by a special carbohydrate callose layer. The tetrads are closely packed along the inner face of the tapetum and each has a prominent callose tip protruded towards the theca of the locule. Microsporogenesis in both the lines is normal up to the formation of
tetrads. In the fertile anther, the microspores are normally released from tetrads, and the latter appear in a line along the inner thin wall of the tapetum. The tapetal cells are parietal in disposition. In some areas, microspores come in contact with degenerating middle wall layer and endothecium. In sterile line, on the contrary, microspores collapse immediately after their release from the tetrad. Unlike in fertile, the tapetum of the sterile line, at this stage, persists, and indicates the striking situation and differences between the fertile and sterile anthers. The wall layers particularly the epidermis and endothecium remain intact during degeneration of sporangial locule. The middle wall layer is completely crushed and degenerates.

**HISTOCHEMISTRY**

**Microsporogenesis in Fertile and Cytoplasmic Male Sterile (CMS) *Raphanus* and *Pennisetum***

**Total insoluble polysaccharides:**

During the early sporogenesis in the fertile lines of *Raphanus* and *Pennisetum*; the epidermis, wall layers, and sporogenous and connective tissues exhibit distinct PAS positive cell walls (Figs. 1,2,4 see PLATE I.A). Among these tissues, connective alone stores plentiful of PAS positive grains to begin with. At a late stage of sporogenous growth similar concentration and distribution of wall polysaccharides was observed both in fertile and sterile lines. However, an increase and accumulation of PAS positive grains was observed in the wall layers, especially the middle ones in addition to that of connective tissue (Figs. 2,10,11,24,25 see PLATE II.A). When sporogenous cells are transformed into meiocytes (PMCs) synthesis of polysaccharides increases in the wall layers and also connective. The meiocytes contain only wall polysaccharides (Figs. 3,4,12). At times a few PAS positive grains were observed in the fertile anthers. Similarly, in sterile line, anther tissues also show positive reaction showing the presence of polysaccharides. But quantitatively the number of PAS grains appears to be low.
In the latter when compared to situation in the fertile line (Figs. 17, 25, 26). When PMCs begin meiosis, an additional wall polysaccharides are synthesized and deposited, especially towards the thecal side in *Pennisetum* (Figs. 12, 13 see PLATE II.B). The deposition is almost similar to that of sterile anther (Fig. 26 see PLATE II.E). During dyad and tetrad stages both fertile and sterile lines show only a few PAS grains in the anther wall layers. The cytoplasm of tapetum in general reacts positive PAS test (Figs. 5, 14, 18, 27, 28 see PLATE I.D and PLATE II.C). The radial cell walls of tapetum are very thin and are feebly PAS-positive. The cells of the remaining anther tissues are distinctly PAS-positive. The cell walls of microspores within tetrads are distinctly PAS-positive. The cytoplasm reacts moderately for polysaccharides. The developing microspores and pollen in fertile lines show distinct cell walls. The tapetum normally degenerates in the fertile line of *Raphanus*, (Figs. 6, 7, 8, 9 see PLATE I.B). Whereas in *Pennisetum*, while degenerating, the tapetum shows more of cytoplasmic polysaccharides. Middle wall layers in both the plants gradually degenerate. At maturity PAS storage declines and is lost in the fertile anthers (Figs. 15, 16 see PLATE II.D). In the sterile line of *Raphanus*, the tapetum at tetrad stage begins to enlarge and form a tapetal syncytium exhibiting very poor stain for polysaccharides in the cytoplasm as well as cell walls (Figs. 19, 20, 21 see PLATE I.C, E, F). The endothecium shows a few PAS grains. No hypertrophid development of tapetum is observed in *Pennisetum*, whereas in *Raphanus* the tapetal enlargement progresses. Consequent upon enlargement of tapetum and its hypertrophy, tetrads in *Raphanus* are crushed in the centre of the anther locules. This results in non separation of microspores from tetrads (Fig. 22). Finally, barring epidermis, endothecium, the remnants of the middle wall layers and the inner contents of the locules especially the tapetum; microspore tetrads degenerate (Fig. 22 see PLATE I.G). However in sterile line of *Pennisetum* the situation is marginally different from that of sterile *Raphanus*. The tapetum is normally present adpressed to the parietal region of the anther locule. The microspores show distinct PAS-positive wall. Following the separation of microspores from tetrad,
the microspores begin to shrivel and degenerate (Fig. 29,30 see PLATE II.F). The tapetum which is all the while at parietal position, begins to lose its cell walls and becomes plasmodium, and gradually its content mixes with the developing microspores and degenerates subsequently. The degenerated mass shows feeble stain for PAS (Figs. 30,31 see PLATE II.H). The anther wall layers, particularly the degenerating middle wall layers, show more of PAS stain. The epidermis is a deeply stained layer in the degenerating anther (Figs. 30,31). No PAS positive storage grains are observed during degeneration of anther locule either in the tapetum or in any of the wall layers. In sterile line of *Raphanus*, at collapsed stage, epidermis shows the starch grains (Fig. 23 see PLATE I.II).

**Callose:**

Both *Raphanus* and *Pennisetum* of fertile and sterile lines show additional callose wall deposition around the meiocytes (Figs. 32,34,36,40), as verified by ABF test. From the beginning of meiosis, the meiocytes are enveloped by this additional wall deposition which persists until the meiosis is completed (Figs. 33,35,37,38,39,41). Later, the dissolution of callose wall around the tetrad occurs and the microspores are liberated into the locules showing the deposition of rudimentary wall around each.

**Ascorbic acid (AA):**

In the fertile lines, the early and late sporogenous cells, and meiocytes show considerably rich concentration of ascorbic acid distributed in the cytoplasm and also along with the cell walls. Young tapetum shows higher concentration of AA than the sporogenous cells. The wall layers show considerably low content of ascorbic acid (Figs. 42,43,51,52). Almost similar type of distribution and concentration of ascorbic acid is observed in the sporogenous cells and meiocytes of sterile lines (Figs. 47,55). During meiosis meiocytes also show similar AA distribution. Following meiosis tetrads show moderate distribution of ascorbic acid in both fertile and sterile lines. (Fig. 44 see PLATE V. I)). However, in sterile anthers of *Raphanus* the tetrads contain comparatively high AA content. The
tapetum retains high AA content all through its development (Fig. 48). The microspores in fertile lines of both Raphanus and Pennisetum show greater accumulation of AA (Figs. 45,53). In the sterile line the microspores begin to degenerates showing low concentration of AA (Figs. 49,56,57 see PLATE V. E,F). The tapetum in sterile line of Raphanus shows very low AA content when compared to CMS of Pennisetum in which AA is considerably high. The tapetum in sterile line of Raphanus develops syncytium having a very low AA content (Fig. 49). At pollen grain stage, tapetum or fertile anther undergoes degeneration exhibiting high content of AA (Fig. 46). In sterile line, the collapsed degenerating locule shows moderate AA content (Fig. 50). In Pennisetum, fertile microspores and degenerating tapetum contain moderate AA content (Figs. 53,54). Similarly in sterile line these exhibit moderate AA content (Figs. 56,57). Anther wall layers all through the anther development react uniformly to AgN03 test showing moderate amount of AA content (Fig. 58).

Ribonucleic acid (RNA)

In both fertile and sterile lines during the anther development from sporogenous tissue to meiocytes stages, the staining intensity for RNA is almost uniform. The sporogenous tissue shows rich staining for RNA, whereas the tapetum denotes higher staining in the cytoplasm and nucleoli (Figs. 59,62,71,72 see PLATE III.A and PLATE IV.D). At prophase, slight declining trend is noticed in the PMCs (Figs. 72,77 see PLATE IV.D). During meiosis dyads and tetrads of both fertile and sterile lines show rich RNA content in the cytoplasm and nucleoli, whereas tapetum reacts strongly to RNA test (Figs. 60,63,73,74,78 see PLATE IV.E). In the fertile line of Raphanus during post-meiotic stage pollen grains show rich RNA both in cytoplasm and nucleioli (Fig. 61 see PLATE III.B). The degenerating tapetum has moderate content of RNA (Fig.61). In its sterile line, degenerating microspores have low RNA tinge in the cytoplasm (Figs. 64,65). The tapetal syncytium, however, possess higher concentration of cytoplasmic and nucleolar RNA (Figs. 66,67). The same concentration continues until syncytium

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undergoes degeneration (Figs. 64, 65, 66, 67 see PLATE III.C). The degenerated mass of anther locule finally reacts strongly for RNA content (Fig. 70 see PLATE III.D). In the fertile line of *Pennisetum* the microspores during mid-vacuolate period show reduced content of cytoplasmic RNA, but nucleolar staining being high in them (Fig. 75 see PLATE IV.B). The degenerating tapetum in this line has high cytoplasmic RNA (Fig. 75), while in the sterile one degenerating microspores and the surrounding persistent tapetum exhibit very low RNA content (Figs. 79, 80, 81 see PLATE IV.F, G). This situation is unlike that of *Raphanus* in which the degenerated mass reacts strongly for RNA. In the fertile line of *Pennisetum*, pollen grains are very rich in cytoplasmic RNA (Fig. 76 see PLATE IV.C). The degenerated mass in the anther locule of sterile *Pennisetum*, very low cytoplasmic RNA is observed (Fig. 82 see PLATE IV.H). The endothecial layer shows negligible RNA content (Figs. 80, 81).

**DNA**

In the fertile and sterile lines of *Raphanus* and *Pennisetum*, DNA stainability in the sporogenous cells and tapetum is considerably high in their nuclei (Figs. 83, 88). In the meiocytes, Feulgen stainability to some extent, is reduced in the nuclei (Fig. 89). In the dyads and tetrads of both fertile and sterile lines, the nuclei show low stainability for DNA (Figs. 84, 86, 90). The tapetal nuclei at all stages on the contrary react comparatively high for DNA (Figs. 84, 86, 90). The microspores in the fertile line of *Raphanus* has low Feulgen stainability, whereas the tapetum reacts with the test for rich DNA at this stage (Fig. 85). In the sterile line of *Raphanus*, degenerating locular mass shows almost no DNA content in the locule (Fig. 87). However, the degenerating tapetal nuclei become shapeless mass showing rich staining. In the sterile line of *Pennisetum*, degenerating microspores show poor reaction for Feulgen stain (Figs. 91, 92). The tapetum on the contrary becomes binucleate and the nuclei react strongly for DNA (Figs. 91, 92).
Total proteins:

Both fertile and sterile lines, in the early and late stages of sporogenous tissues and tapetum show high cytoplasmic and nuclear proteins. The tapetum reacts comparatively more positive to proteins than the sporogenous tissue (Figs. 93,100, 110,115,116). Meiocytes retain almost same protein content (Figs. 94,95). The tapetum at the beginning of the meiosis maintains high protein content. In the meiotic derivatives of dyads and tetrads of the fertile and sterile lines protein content is high (Figs. 96,101,111,117 see PLATE V.A). In *Raphanus* protein content in the separated microspores is high in the nuclei (Figs. 97,102,104). The tapetum in fertile lines of *Raphanus* and *Pennisetum* consistently shows high proteins both in the cytoplasm and in the nuclei (Figs. 97,98,112 see PLATE V.B). In the sterile line of *Raphanus* the syncytium proliferates and fill the anther locules exhibiting high protein content (Figs. 103,104,105,106,107 see PLATE V.C). In the sterile line of *Pennisetum* no syncytium occurs. During degeneration, both tapetum and microspores show high protein content (Figs. 118,119,120). Rarely, in *Raphanus*, formation of hypertrophiad tapetum and degeneration of microspores was observed. The hypertrophiad tapetum has conspicuous nuclei and dense cytoplasm both being high in protein content (Fig. 108). The pollen of fertile line however, shows higher protein content similar to the degenerating tapetal tissue. The endothelial and middle wall layers, however, are not rich in proteins (Fig. 99). In the sterile line of *Raphanus*, the collapsed locular mass shows rich protein content, whereas the anther wall layers show negligible tinge (Fig.109). In the fertile line of *Pennisetum* the microspores and tapetum are rich in proteins. In the former however, nuclear proteins are conspicuous than those in the cytoplasm (Figs. 112,113). In the sterile line persistent tapetum and degenerating microspores show reduced protein content (Figs. 118,119,120). While the fertile pollen grains are rich in proteins (Fig. 114), the degenerating locular mass in the sterile line also shows rich protein content (Fig. 121).
HISTOENZYMOLOGY

Dehydrogenases:

Following dehydrogenases are localized in the fertile and sterile anthers of *Raphanus* and *Pennisetum*:

1. Glucose - 6 - Phosphate dehydrogenase;
2. Succinate dehydrogenase;
3. Malate dehydrogenase;
4. Glutamate dehydrogenase;
5. Isocitrate dehydrogenase.

In fertile and sterile anthers of *Raphanus* and *Pennisetum*, sporogenous tissue and meiocytes react considerably for high content of glucose-6-phosphate dehydrogenase (Figs.122,125,128,131), whereas activities in them for succinate dehydrogenase (Figs.134,137,141,142,145 see PLATE IV.C), Malate dehydrogenase (Figs.148,154,157), Glutamate dehydrogenase (Figs.160,163,166) and isocitrate dehydrogenase (Figs.172,174,178,179) are moderate. The tapetum, however, shows higher activity for these enzymes. Following meiosis, the dyads, tetrads and tapetum show equally higher activities for glucose-6-phosphate dehydrogenase (Figs.123,126,129,132 see PLATE VI.A), succinate dehydrogenase (Fig.143), Malate dehydrogenase (Figs.149,151,155,158), glutamate dehydrogenase (Figs.167,170) and isocitrate dehydrogenase (Figs.173,175). In the fertile line of *Raphanus*, the activities for localizing succinate dehydrogenase (Figs.135,137,138,149) and glutamate dehydrogenase (Figs.161,164) are higher in the tetrads than in the sterile ones. The tapetum in fertile anther has higher content of these than the sterile one. During post-meiotic period, the activities of glucose-6-phosphate dehydrogenase (Figs.124,130,132 see PLATE VI.B), succinate dehydrogenase (Figs.136,139,144), malate dehydrogenase (Figs.150,152,156), glutamate dehydrogenase (Figs.162,168,170) and isocitrate dehydrogenase (Figs.180,181) are generally moderate in the microspores. The intensity is confined to the microspore and pollen walls. The nuclear area is also rich in enzymes activity. The tapetum at this stage undergoes degeneration, showing
variation in the activities from moderate to rich levels. The epidermis, endothecium and middle wall layers show minimal activities for the said enzymes. However, in the fertile line *Raphanus*, the activity of succinate dehydrogenase is high in microspores and tapetum, while in its sterile line, the tapetal syncytium shows minimum activity. The degenerating microspores exhibit very low content (Figs. 136, 139). In *Pennisetum*, the activity of succinate dehydrogenase is low in the degenerating microspores of sterile line (Fig. 146). In the sterile line of *Raphanus* the activity for isocitrate dehydrogenase in the tapetal syncytium is very minimal, but moderate in the degenerating microspores (Fig. 176). The degenerating mass in the locule in general exhibits higher activity for these enzymes (Figs. 127, 133, 140, 147, 153, 159, 165, 171, 177, 182 see PLATE VI.D,E).

Non-specific Esterases:

In the fertile and sterile anthers of *Raphanus*, the sporogenous tissue shows moderate enzyme activity (Figs. 183, 185). In the tapetum, however, the activity is rich. The esterase activity is strong in pollen grains and tapetum (Fig. 184 see PLATE VII.A). In the sterile line, the activity of esterase is high in the tapetum and tetrad (Fig. 186). During the post meiotic period, the young and developing microspores become elipticle exhibiting moderate activity (Figs. 187, 188). In the collapsed anther locule, particularly the degenerating mass, reacts strongly for esterases (Fig. 189 see PLATE VII.B). In *Pennisetum* at post-meiotic period, pollen show high activity in their walls and moderate in the cytoplam. The degenerating tapetal tissue and middle wall area of anther show high activity (Fig. 190). However, in the sterile line, wall layers and tapetum do show low activity. The degenerating mass however, reacts strongly for esterases (Fig. 191).

Phosphatases:

The following phosphatases are localized in both fertile and sterile anthers of *Raphanus* and *Pennisetum*:

1. Acid phosphatase;
2. Alkaline phosphatase;
3. Adenosine triphosphatase (ATPase).

The sporogenous tissue and meiocytes in both fertile and sterile anthers of *Raphanus* and *Pennisetum* do not exhibit rich activity for acid phosphatase (Figs. 192, 195, 199, 201 see PLATE VII.C), alkaline phosphatase (Figs. 211, 213) and ATPase (Fig. 219). The middle wall layers at this stage, however, exhibit high activity for acid phosphatase and alkaline phosphatase, but low for ATPase. In the fertile anthers of both *Raphanus* and *Pennisetum*, the tetrads show high activity for acid phosphatase (Figs. 193, 196). But, in the corresponding stages of sterile anthers, dyads and tetrads show moderate activity for these enzymes (Figs. 202, 208, 214). During the post meiotic period in the fertile anther of *Raphanus*, acid phosphatase and alkaline phosphatase are gradually reduced in the middle wall layers and in the normal pollen grains, a gradual increase in the activity of these enzymes was observed particularly in the pollen walls (Figs. 194, 207 see PLATE VII.D). However, in the sterile anthers the degenerating microspores, tapetal syncytium and middle wall layers show low activity for acid phosphatase (Fig. 197), alkaline phosphatase (Fig. 209) and ATPase (Figs. 216, 217 see PLATE VII.F). In the fertile anthers of *Pennisetum* during post meiotic phase, strong activity for acid phosphatase (Fig. 200), alkaline phosphatase (Fig. 212) and ATPase (Fig. 220) was observed in the middle wall layers, tapetum and walls of microspores. However, in sterile anthers the middle wall layer and tapetum show strong activities for acid phosphatase (Figs. 203, 204, 205) and alkaline phosphatase (Fig. 214), but negligible for ATPase (Fig. 221). In sterile anthers of both *Raphanus* and *Pennisetum*, the degenerating locular mass generally exhibits strong activities for acid phosphatase (Figs. 198, 206), alkaline phosphatase (Figs. 210, 215 see PLATE VII.E) and ATPase (Figs. 218, 222 see PLATE VII.G).

**Oxidases:**

The following enzymes are localized in fertile and sterile anthers of *Raphanus* and *Pennisetum*:
1. Peroxidase;
2. Cytochrome Oxidase.

In both fertile and sterile lines of *Raphanus* and *Pennisetum*, the sporogenous tissue and meiocytes react feebly to peroxidase (Figs. 223, 231) and moderate to cytochrome oxidase tests (Figs. 235, 240, 241). However, in the anther wall layers their activities are considerably high. In fertile anthers of *Raphanus* and *Pennisetum* at dyads and tetrads the activity of peroxidase (Figs. 224, 229) is strong, but moderate in the dyads of sterile lines (Fig. 226). In the tapetum and middle wall layers, the cytochrome oxidase activity is high (Figs. 237, 242 see PLATE VIII.F,G). In the fertile anther of *Raphanus*, the pollen grains show strong activity for peroxidase (Fig. 225 see PLATE VIII.A,B) and also for cytochrome oxidase (Fig. 236 see PLATE VIII.E) especially in the pollen walls. Whereas in the sterile line, the degenerating microspores show minimal activity for peroxidase, the middle wall layer shows feeble activity for this enzyme (Fig. 227 see PLATE VIII.C) and cytochrome oxidase (Fig. 238). Similarly, the tapetal syncytium and middle wall layer show feeble activity for cytochrome oxidase. The activity of these enzymes is generally observed high in the degenerating mass of microspores and also in the adjoining tapetum (Figs. 228, 239 see PLATE VIII.D,H). In the fertile line of *Pennisetum*, the activities of peroxidase (Fig. 230) and cytochrome oxidase (Fig. 243) are moderate in the developing microspores. The degenerating tapetal tissue and middle wall region of anther show high enzyme activities. But in sterile line, negligible activity of these enzymes was observed in degenerating mass of microspores. The persistent tapetum, however, shows strong reaction for peroxidase (Fig. 232) and cytochrome oxidase (Fig. 244). In the collapsed anther locule, the degenerated mass reacts strongly for these (Figs. 233, 234 and 245).

**Lipids:**

In the anthers of fertile line, lipids are high in the sporogenous tissue and PMCs, whereas in sterile anthers, the substance appears moderate. The wall layers
and tapetum in both these lines react strongly for lipids (Figs. 246, 247, 249). During meiosis, the dyads and tetrads show high lipid content (Figs. 250, 254, 256 see PLATE VIII.I). In the fertile anther of *Pennisetum*, the sporogenous tissue and PMCs show moderate concentration of lipids. The wall layers and tapetum react strongly for the substances (Fig. 253). In the fertile anthers of *Raphanus*, however, the microspores and pollen possess high lipid content (Fig. 248). Similarly, in the sterile line the microspores show high lipid content before their degeneration (Fig. 251). On the contrary, the tapetum in sterile anther shows minimum quantity of lipids. The fertile pollen of *Pennisetum* are rich in lipids. The wall layers and tapetum show moderate activity (Fig 255). In the sterile line degenerating microspores however, show moderate content of lipids (Figs. 257, 258, 259, 260), while these bodies are minimal in tapetum (Fig. 260). The degenerating mass of microspores and tapetum in the locules of both *Raphanus* and *Pennisetum* reacts very strongly for lipids (Figs. 252, 261).

**BIOCHEMICAL (Electrophoresis)**

Polyacrylamide gel electrophoresis tried on for anther proteins in normal and cytoplasmic male sterile lines of *Raphanus* and *Pennisetum* showed differences in their protein profile. The fertile lines of both these plants showed certain additional protein bands when compared with the protein profiles of sterile anthers. This observation is in line with the synthesis of gametophytic proteins at anthesis. On the other hand, as the microspores in both CMS lines aborted, the synthesis of some proteins is affected, as evidenced in photograph by arrow marks (Figs. 262, 263).
**Raphanus sativus**

Sections of fertile anther.

Figs. 1-9: Localization of total insoluble polysaccharides in anther sections during microsporogenesis.

(Ct = connective; E = Endothecium; M = Meiocytes; Ms = Microspores; Pg = Pollen grain; Sp = sporogenous cells; T = Tapetum; W1 = Wall layer).

Fig. 1: Sporogenous cells possess distinct PAS positive cell walls.

Fig. 2: Sporogenous tissue. Note starch grains present in the wall layer and connective.

Fig. 3: PMC’s shows distinct PAS- positive cell walls.

Fig. 4: Note abundant persistence of starch in the wall layers and connective at the beginning of meiosis.

Fig. 5: Tetrad stage with distinct glandular tapetum.

Fig. 6: Young microspores. Note starch grains in microspores are being utilized.

Fig. 7: Microspores with disintegrating tapetum.

Fig. 9: Mature pollen grains showing starch storage.

Note PAS - positive endothecial thickenings.
*Pennisetum typhoides*

Sections of fertile anther.

Figs. 10-16: Localization of total insoluble polysaccharides during microsporogenesis of fertile anther.

(DD = Dyad; Ml = Middle wall layer; Ms = Microspores; Pg = Pollen grain; Sp = Sporogeneous; T = Tapetum).

Figs. 10-11: Sporogenous cells and early meiocytes. Note starch in the middle wall layer.

Fig. 12: Meioocytes react strongly to PAS test.

Fig. 13: At late meiocyte stage, showing asymmetrical deposition of callose wall.

Fig. 14: Dyad stage. Note persistence of starch in the middle wall layers.

Fig. 15: Note peripheral arrangement of microspores around the locule.

Fig. 16: Pollen grains react strongly to PAS test.
**Raphanus sativus**

Sections of CMS anther.

Figs. 17-23: Localization of total insoluble polysaccharides during microsporogenesis.

(Ct = Connective; Ht = Hypertrophoid tapetum; M = Meiocytes; Ms = Microspores; Sy = syncytium; Sp = sporogenous cells; T = Tapetum; Wl = wall layers).

Fig. 17: Meiocytes show distinct PAS-positive cell walls.
   Note small starch grains in the wall layers.

Fig. 18: Tetrad stage. Note increase in starch grains in wall layers.
   Connective stores plentiful of starch.

Fig. 19: At microspores. Note decline of starch grains in the connective.
   Tapetum is becoming syncytium.

Fig. 20: Stages showing abortion of microspores and also the disintegration of syncytum. Note feeble stain for PAS.

Fig. 21: At late meiocytes showing hypertrophied tapetum.

Fig. 22: The same as above at tetrad stage. Note, The remnants of diminishing small starch grains in wall layers.

Fig. 23: Old collapsing CMS anther, feebly PAS positive.
*Pennisetum typhoides*

Sections of CMS anther.

Figs. 24-31: Localization of insoluble polysaccharides during microsporogenesis.  
(DD = Dyad; Dm = Degenerating microspores; M = Meiocytes;  
Ml = Middle wall layer; Sp = sporogenous; Tt = Tetrad;  
Pt = Persistent tapetum).

Figs. 24-25: At sporogenous cells and early meiocytes. Note starch deposition in  
the middle wall layer.

Fig. 26: At meiocytes. Note distinct PAS positive walls.

Figs. 27-28: Dyad and tetrad stages respectively. Note very small starch grains in  
wall layers.

Figs. 29-30: At gradual abortion of microspores strong PAS- positive reaction  
between the tapetum and middle wall layer cell is seen. Note  
persistent tapetum feebly positive.

Fig. 31: At pollen abortion stage showing feeble stain in the degenerated mass.
Localization of callose during microsporogenesis of *Raphanus sativus* and *Pennisetum typhoides*

Figs. 32-33 : *Raphanus* fertile anther.

Figs. 34-35 : *Raphanus* sterile anther.

Figs. 36-37 : *Pennisetum* fertile anther.

Figs. 40-41 : *Pennisetum* sterile anther.

Figs. 32,33 : Note callose deposition around meiocytes and tetrad respectively.

Figs. 34,35 : Note callose deposition around the meiocytes and tetrads, respectively.

Fig. 36 : During late PMCs stage the deposition of callose is seen more towards the center of the locule.

Figs. 37,38 : Deposition of callose during dyads and tetrads.

Fig. 39 : Note gradual distribution of callose around the microspores.

Fig. 40 : Deposition of callose during late PMCs towards the center of the locule.

Fig. 41 : Callose deposition during dyad.
**Raphanus sativus**

Sections of fertile and sterile anther.

Figs. 42-50 : Localization of ascorbic acid during microsporogenesis.

Figs. 42-46 : Fertile anther.

Figs. 47-50 : Sterile anther.

(M = Meiocytes; Pg = Pollen grain; Sy = Syncytium; Sp = Sporogenous cells; T = Tapetum; Tt = Tetrads; V = Vacuole).

Fig. 42 : Sporogenous tissue and tapetum show rich ascorbic acid. Wall layers show a few small AA grains.

Figs. 43 : Early meiotic stage. Meiocytes and tapetum show high content of ascorbic acid.

Fig. 44 : Tetrads showing depletion of ascorbic acid, but tapetum retain high ascorbic acid in the tapetum.

Fig. 45 : Note Increase in ascorbic acid content in the tapetum and microspores.

Fig. 46 : Pollen grains show high ascorbic acid content.

Fig. 47 : Meiocytes and tapetum show rich ascorbic acid content.

Fig. 48 : In the tetrads ascorbic acid is increased whereas in tapetum it is rich.

Fig. 49 : Microspores and vacuolate tapetum show decline in ascorbic acid content. Note Microspore walls react for ascorbic acid.

Fig. 50 : Abortive locule showing high AA.
Pennisetum typhoides

Section of fertile anther.

Figs. 51-58 : Localization of ascorbic acid (AA) during microsporogenesis.
Figs. 51-54 : Fertile anther.
Figs. 55-58 : Sterile anther.

(E = Endothecium; M = Meiocytes; Ml = Middle wall layer; Ms = Microspores; Sp = Sporogenous cells).

Fig. 51 : Note ascorbic acid is distributed more along the cell walls of sporogenous cells, tapetum and wall layers.
Fig. 52 : Note meiocytes wall show reduced ascorbic acid in the cytoplasm.
Fig. 53 : Mid vacuolate microspores stage. Note minute ascorbic acid grains in the tapetum along the walls of anther.
Fig. 54 : Microspores regain ascorbic acid grains. Note around nuclei and inner endothecial walls.
Fig. 55 : Meiocytes, tapetum and wall layers show ascorbic acid, greatly along the cell walls.
Fig. 56-57 : Degenerating microspores showing low ascorbic acid content.
Fig. 58 : Abortive locular mass rich in AA.
Raphanus sativua

Sections of fertile and sterile anthers.

Figs. 59-70 : Localization of RNA during microsporogenesis.

Figs. 59-61 : Fertile anther sections.

Figs. 62-70 : Sterile anther sections.

(Cm = Collapsing microspores; M = Meiocytes; Pg = Pollen grain; Sp = Sporogenous cells; T = Tapetum; Sy = Syncytium).

Fig. 59 : Meiocytes and tapetum show rich cytoplasmic RNA and their nucleoli react strongly.

Fig. 60 : Late tetrads showing decline in RNA content. Note tapetum is rich in RNA content.

Fig. 61 : Pollen grains are rich in RNA. Tapetum is undergoing degenerating.

Fig. 62 : Sporogenous tissue showing high RNA.

Fig. 63 : Tetrads and tapetum both showing rich cytoplasmic RNA.

Figs. 64,65 : Microspores and tapetum showing reduced content of cytoplasmic RNA.

Fig. 66 : Syncytium showing high RNA content, but low in collapsing microspores.

Fig. 67 : Hypertrophied tapetum at microspores stage showing high RNA.

Fig. 68 : Hypertrophied tapetum high in RNA

Fig. 69 : Hypertrophied tapetum having large vacuoles and high RNA.

Fig. 70 : Abortive anther locale showing high RNA.
Pennisetum typhoides

Sections of fertile anther

Figs. 71-76 : Localization of RNA during microsporogenesis.
(DD = Dyad; Ms = Microspores; Pg = Pollen grain;
Sp = Sporogenous cells; TT = Tetrad; Wl = Wall layers).

Fig. 71 : Early sporogenous tissue and wall layers showing moderate RNA.

Fig. 72 : Late sporogenous tissue showing an increase in cytoplasmic RNA.

Figs. 73,74 : Dyad, tetrad and tapetum all show high RNA staining.

Fig. 75 : Vacuolate microspores showing much reduced cytoplasmic staining
and degenerating tapetum possess still high RNA.

Fig. 76 : Shedding pollen show very high RNA.
Pennisetum typhoides

Sections of sterile anther

Figs. 77-82: Localization of RNA during microsporogenesis.

(Cm = Collapsing microspores; Dd = Dyad; Dm = Degenerating microspores; E = Endotheium; Sp = Sporogenous cells).

Fig. 77: Sporogenous tissue and wall layers show moderate cytoplasmic RNA.

Fig. 78: Dyads and tapetum show Strong RNA staining.

Figs. 79-81: Degenerating microspores show low RNA content.

Fig. 82: Abortive locule.
Raphanus and Pennisetum


Figs. 83-85: Raphanus sterile anther section.

Figs. 86-87: Raphanus sterile anther section.

Figs. 88-89: Pennisetum fertile anther section

Figs. 90-92: Pennisetum sterile anther section

(M = Meiocytes; Ms = Microspores; Pg = Pollen grain; Sp = Sporogenous cells; T = Tapetum; Tt = Tetrad; W1 = Wall layers).

Fig. 83: Sporogenous cells show moderate feulgen staining in their nuclei.

Fig. 84: Microspore tetrads show low staining for DNA in their nuclei. Note intense staining in tapetal nuclei.

Fig. 85: Microspores showing low feulgen staining for DNA in their nucleus.

Fig. 86: Microspore tetrads show relatively low content of DNA than the tapetal cells.

Fig. 87: Abortive microspore nuclei show very low content of DNA.

Fig. 88: PMC's show low feulgen stainability in their nuclei than the tapetal nuclei.

Fig. 89: Microspores and tapetum show high content of DNA.

Fig. 90: The nuclei of the dyad cells show comparatively low DNA content than the tapetal nuclei.

Figs. 91,92: Degenerating microspores show low DNA content in their nuclei.
Raphanus sativus

Sections of fertile anther

Figs. 93-99: Localization of total proteins during microsporogenesis.

(M = Meiocytes; Ms = Microspores; Sp = Sporangious cells;
T = Tapetum).

Figs. 93-95: Tapetum and meiocytes showing higher nuclear proteins.

Fig. 96: Note, both tetrads and tapetum show increase in proteins synthesis.

Fig. 97: Young microspores show high nuclear proteins than the cytoplasm.

Fig. 98: Note decline in proteins in the microspores but not in tapetum.

Fig. 99: Mature pollen grains prior to dehiscence show high protein content.