I wish to express my deep sense of gratitude to the members of the executive council of the Indian Botanical Society for very kindly electing me for this prestigious award named after a great botanist Dr. Birbal Sahni. Although I was not fortunate to have met Professor Sahni I had the privilege of having some training in Paleobotany at the prestigious BIRBAL SAHNI INSTITUTE. For this award lecture I have chosen to speak on ‘Tapetum’ on which I have been working for nearly 43 years.

Tapetum is the inner most layer of the anther wall surrounding the microsporogenous cells. The tapetum is secretory or glandular in some species and amoeboid or plasmodial in others.

Tapetum holds considerable physiological significance. It is a nutritive jacket through which all the nutrients required by sporogenous cells pass. The importance of tapetum is demonstrated by the male-sterility resulting from any lesion in tapetal biogenesis.

**TAPETAL FUNCTIONS:**

The functions of tapetum as illustrated by Fig. 1 are varied.

1. **NUTRIENTS:**

A variety of chemical compounds, namely reducing sugars, amino acids, lipids, pollenkitt, tryphine and number of proteins pass unmodified through the tapetal cells from the cells of the connective and middle layers through into the microsporangium. The range of compounds actually elaborated within the tapetum itself is unknown.

It is well established that pollen libraries contain a high proportion of cDNAs cognate to pollen-specific or pollen expressed transcripts. Several experiments with anther-specific cDNAs by *in situ* hybridization have shown that the corresponding transcripts are localized within the tapetum in all cases. The question arises that why should tapetal messages dominate the mRNA population of the young anther when several other cell types, including the sporocytes and their derivatives, are present within the microsporangium? It is likely that during early anther development the tapetum is the transcriptionally dominant cell-type either in terms of the number of different transcripts produced, the quantity of individual mRNA species or both.

The results of large number of experiments suggest that TAPETUM produce a number of highly expressed mRNAs. Thus, what ever the function of the corresponding genes, the protein products they specify are evidently required in quantity e.g. structural components of the pollen wall such as sporopollenin, enzymes like B (1,3)-glucanase and a large supply of simple nutrients. In contrast to the tapetum, the sporogenous cells do not synthesize the battery of highly abundant cell-
specific mRNAs.

Changes in the constitution of the pool of tapetum-specific transcripts presumably reflect the ontogeny of the tissue. The tapetum-specific transcripts, A3 and A9 which are the earliest anther-specific messages described, appear at archesporial cell stage only for a short time after the differentiation of tapetum and sporogenous cell lineages. Other tapetum-specific transcripts, e.g. 92b and TA29 are found in high concentrations in the anthers as tapetum passes through middle age, but fall gradually to undetectable level with the degeneration of tapetum. This suggests that the tapetum begins producing certain highly abundant and cell-specific transcripts soon after its differentiation, and continues doing so throughout its lifetime, ceasing only when its degeneration is under way. It is likely that some tapetal genes are not regulated as a consequence of tapetal degeneration, a process that may be determined by tapetum specific genes (Scott et al., 1991). The transcriptional activity of the pre-meiotic anther appears dominated by the tapetum. Tapetum continues to produce a range of highly abundant and cell-specific transcripts even after the completion of meiosis. The post-meiotic mRNA pool now contains abundant transcripts derived from the microspores and from several other cell types within the anther wall.

2. CALLASE ENZYME:

The tapetal cells synthesize an enzyme complex of endo- and exo- (1,3)-glucanase (callase) synthesized by the tapetal cells and secretes it in the anther locule. The enzyme is critical for degradation of callose wall present around the microspore tetrad. In this way the enzyme is responsible for release of microspores from the common callose wall around the tetrads. During prophase I of meiosis, the meiocytes secrete a complete wall of callose, a $\beta$ (1,3)-glucan, between the plasmalemma and the original cellulotic wall (Heslop-Harrison, 1968). There exists a strong relationship between locule pH, callase activity and the timing of microspore release. Any deviation in this cycle leads to faulty dissolution of callose ($\beta$ (1,3)-glucan).

Recently, we have observed that premature callose wall dissolution from some microspore tetrads of the anthers of ethrel or ethaphon (2-chloroethyl phosphonic acid) treated plants of cotton (Gossypium arboreum) leads to the formation of non-viable pollen grains with thin exine devoid of characteristic spines. On the other hand, in the same locule, the callose wall degenerated at the appropriate stage and the non-viable pollen grains developed with exine with more or less normal spines (Fig.2 a-e).

Fig.2 a-c TEM. part of anthers of Gossypium arboreum plants treated with different concentrations of ethrel. Fig.2 d-e. TEM photographs of pollen grains of ethrel treated plants. a. Showing pre-mature dissolution of callose wall from microspore tetrad (Mt) 840X, b. showing non-viable pollen grains both with (Se) and without spines (Te) 640X, c. showing eight non-viable pollen grains four of which are with spine (Se) and four are thin walled (Te) with intact tapetum 640X, d. non-viable pollen with spine 2100X and e. non-viable pollen without spine 1600X.
3. MEIOSIS:

The fact that microspore mother cells are capable of developing into pollen grains in culture, free from the influence of tapetum, but only when they have reached leptotene--zygotene stage of meiosis, provides compelling evidence that meiosis is triggered by some stimulus originating outside the sporogenous cells (Ito and Stern, 1967).

4. SPOROPOLLENINS (C₉₀H₂₀Oₐ):

Sporopollenins, which are oxidative polymers of carotenoids and carotenoid esters, are probably the most resistant organic materials of direct biological origin found in nature. They make up the outer wall of pollen grains, spores and many microorganisms. The cellulosic primexine outside the plasmalemma of the young microspores provides the matrix of receptors on which protosporopollenin or sporopollenin is deposited. During the initial phase of wall growth, sporopollenin is formed from precursors located within the microspore cytoplasm and secreted outside the cell between the plasma membrane and the callose wall. However, in most species the bulk of exine develops from the sporopollenin secreted by the tapetal cells after the release of microspores from the common callose wall. Since exine deposition commences while the microspores are held in the tetrad, enzymes polymerizing this early phase of wall deposition are likely to originate from the microspore itself. During the post-tetrad phase of wall growth, the tapetum also contributes enzymes responsible for the synthesis of the carotenoid precursors of sporopollenin present within the tapetum and the microspores.

5. ENDOTHECIUM DEVELOPMENT:

Tapetum controls development of endothecium throughout the major course of anther development. This is perhaps done by the production of an inhibitor. After tapetal degeneration production of this inhibitor ceases and fibrous thickenings appear on the radial walls of the endothecial cells (DeFossard, 1969). Another part of this hypothesis is that the tapetum degenerates only when the synthesis of sporopollenin ceases.

DeFossard’s (1969) has proposed the concept of programmed control of tapetum on the development of endothecium. This is supported by our observations on large number of plants exhibiting pollen sterility caused by various factors (Chauhan, 1977a; 1979a; 2001; Chauhan et al., 1982 and Chauhan and Gupta, 2006): In the anthers of male sterile plants where sterility is caused by either generational or environmental factors or is induced by various other methods the malformed tapetum not only results in pollen abortion but also in the inhibition of endothecial development. It emerges from these observations that malformed tapetum produces some inhibitor, which inhibits endothecial development in the anthers of sterile plants. This is strongly corroborated by the fact that in the anthers of ethrel treated Vicia faba plants; the tapetum degenerates more or less completely at sporogenous tissue stage. The endothecial cells in such anthers elongate radially in a manner similar to that of mature anthers except that they lack fibrous bands may be due to the presence of reminiscent of tapetum (Fig.3a & b). It is believed that the complete degeneration of tapetum in such anthers might have led to the formation of characteristic thickenings as well. This is supported by the presence of radially elongated endothecial cells with fibrous thickenings in the anthers at pollen grain stage after complete degeneration of tapetum (Fig.3c).

Our findings also lend support to DeFossard’s (1969) proposition that tapetal cells degenerate only when they cease to synthesize sporopollenin (Fig. 4a-d).

This is evident from the fact that the
exine formation in sterile pollen grains is not much influenced. The malformed tapetum in such anthers is present in one form or the other and seems to continue the production of the inhibitor due to which endothelial development is inhibited (Fig.5 a,b).

6. ANther SPECIFIC GENES

The genes which express exclusively in the anther are known as the anther specific genes (Table 1). Some of these anther specific

**Fig. 3 a-c.** T.S. of anthers of *Vicia faba* plants treated with different concentrations of ethrel. a. Anther showing partially degenerated tapetum (Ta) at sporogenous tissue stage and radially elongated endothelial cells (En) without fibrous bands 280X. Fig. b showing enlarged view of one lobe of the anther shown in Fig.a, arrow shows the radially elongated endothelial cells without fibrous thickenings 480X, c. anther lobe at pollen grain stage showing complete degeneration of tapetum and fibrous bands in the radially elongated endothelial cells (arrow) 480X.

**Fig. 4a-d:** T.S. of anthers of chemically treated plants showing migration of sporopollenin in anther cavity from tapetum to developing microspores. a. T.S. of anther of ethrel treated male sterile plants of *Hordeum vulgare* showing the presence of number of small mitochondria (M) in tapetum and Ubisch bodies at the outer wall of tapetum. 1200X. b. Anther locule showing the presence of Ubisch bodies in surf excel treated plants of *Cicer arietinum*. 2050X. c. T.S. of anthers of 1.5% Surf excel treated plants of *Sesamum indicum* showing Ubisch bodies (ub) secreted by tapetum. 830X. d. Irregular tapetal cells and large number of Ubisch bodies (ub) in manganese deficient plants of *Capsicum annuum* 1500X.

genes have been listed below.

**Behaviour of tapetum during microsporogenesis and microgametogenesis:**

Early stages of differentiation of wall layers do not require elaboration as it is well known that the cells of the primary parietal layer differentiate into a variable number of concentrically arranged layers that envelope the core of sporogenous cells.

A delicate relationship exists between
the tapetum and the microsporogenous cells. Any disturbance in this balance results in the production of non-viable pollen and subsequent male sterility. The importance of tapetum in pollen development is illustrated by the development of a large number of male-sterile mutants due to lesions in some aspect of tapetal biogenesis. Tapetal abnormalities leading to pollen abortion creep up at different stages of anther development. We have recorded following types of tapetal abnormalities leading to pollen abortion:

I. Precocious (pre-meiotic) degeneration and hypertrophying.

II. Post-meiotic persistence, hypertrophying and formation of pseudo-plasmodium.

The above abnormalities result from various factors, some of which are listed below:

1. GENERATIONAL:


2. ENVIRONMENTAL:


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Table: 1 Anther specific genes

<table>
<thead>
<tr>
<th>No.</th>
<th>Anther specific genes</th>
<th>Species</th>
<th>Tissue/cell expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bp</em>1</td>
<td>Brassica</td>
<td>Microspore</td>
</tr>
<tr>
<td>2.</td>
<td><em>Bcp</em>1</td>
<td>Brassica</td>
<td>Tapetum &amp; Microspore</td>
</tr>
<tr>
<td>3.</td>
<td><em>Callase</em></td>
<td>Brassica</td>
<td>Tapetum &amp; Microspore</td>
</tr>
<tr>
<td>4.</td>
<td>LAT51</td>
<td>Tomato</td>
<td>Pollen</td>
</tr>
<tr>
<td>5.</td>
<td>LAT52</td>
<td>Tomato</td>
<td>Pollen</td>
</tr>
<tr>
<td>6.</td>
<td>LAT56</td>
<td>Tomato</td>
<td>Pollen</td>
</tr>
<tr>
<td>7.</td>
<td>LAT59</td>
<td>Tomato</td>
<td>Pollen</td>
</tr>
<tr>
<td>8.</td>
<td>198, 926</td>
<td>Tomato</td>
<td>Tapetum</td>
</tr>
<tr>
<td>9.</td>
<td>T129</td>
<td>Tobacco</td>
<td>Tapetum</td>
</tr>
<tr>
<td>10.</td>
<td>T158</td>
<td>Tobacco</td>
<td>Anther Wall</td>
</tr>
<tr>
<td>11.</td>
<td>Zm13</td>
<td>Corn</td>
<td>Pollen</td>
</tr>
<tr>
<td>12.</td>
<td>Zm38</td>
<td>Corn</td>
<td>Pollen</td>
</tr>
<tr>
<td>13.</td>
<td>SF2, SF18</td>
<td>Sunflower</td>
<td>Anther Wall</td>
</tr>
</tbody>
</table>
b. Sulphur dioxide: Capsicum annuum
(Chauhan and Jain, 1994)

3. DISEASES:

a. Viruses: Cucumber Mosaic Virus in Capsicum
annuum (Chauhan and Srivastava, 1981a; Chauhan et al., 1981),

b. Fungi: Protomyces macrosporous in Coriandrum sativum (Srivastava and Chauhan, 1980).

c. Nematodes: Meloidogyne javanica in Solanum melongena (Chauhan and Srivastava, 1981b), and Capsicum annuum (Bharadwaj and Chauhan, 1983).

d. Insects: Lipaphis erysimi in Raphenussativus (Srivastava and Chauhan, 1979) and Brassica juncea (Singh and Chauhan, In Press) and Aphis spiraeacola in Solanum nigrum (Chauhan et al., 1983).

4. Zinc, Boron and Manganese deficiency: Hydroponically raised Capsicum annuum (Rathi, 2005), Cicer arietinum (Kumar, 2006) and Vicia faba (Parashar, 2006).

3. INDUCED:


b. Gametocides or chemical hybridizing agents: (Abelmoschus esculentus, Allium cepa, Beta vulgaris, Brassica juncea, Capsicum annuum, Cicer arietinum, Cymopsis tetragonoloba, Datura alba, Gossypium arboerum, G. hirsutum, Helianthus annuus, Hordeum vulgare, Lens culinaris, Linum usitatissimum, Lycopersicon esculentum Nicotiana tabacum, Ranunculus muricatus, Raphanus sativus, Sesamum indicum, Solanum melongena, Vicia faba (Chauhan and Singh, 2006; Chauhan, 1976; Chauhan and Kinoshita, 1980b; 1980c; 1980d; Chauhan and Kumar, 1980; Chauhan, 1981; Chauhan, 1986; 1990; Singh et al., 1989; Singh and Chauhan, 2004; Chauhan and Chaudhary, In Press; Chauhan and Chauhan, 2005; Agnihotri and Chauhan, 2004; Chauhan and Agnihorti, 2005a; b; Chauhan and Gupta, 2006).

ULTRASTRUCTURAL BEHAVIOUR OF TAPETUM:

The description under this head is limited to the differences in the ultrastructure of tapetum of the male fertile and CMS lines of Beta vulgaris. The tapetal behaviour in both male fertile and CMS plants is alike up to the microspore tetrad stage. At this stage, the tapetal cells of sterile anthers show some important changes in mitochondria; their cristae show signs of degeneration. In tapetal cells of fertile plants Ubisch bodies appear at the inner tangential walls. In the tapetum of sterile anthers they are absent. At late vacuolate microspore stage, several lipid-like droplets are seen on the outer tapetal cell membrane in fertile anthers, which are lacking sterile ones. Sterile pollen grains are devoid of intine and their exine formation is also inhibited as is evident by the absence of tectum. The most striking difference recorded in the tapetal cells of CMS plants is the total collapse of mitochondria (Nakashima, 1975; Chauhan, 1988).

The mitochondrial genome (mtDNA) of MF and CMS sugar beet plants also show distinctive differences. The mtDNA isolated from normal (N) and male sterile (S) cytoplasms of sugar beet strains TK81-O and TK81-CMS and examined under electron microscope has revealed that mtDNA is composed of a heterogeneous population of circular molecule. Their contour lengths varied between 0.28 and 51 μm. No significant difference has been observed in the distribution of molecular classes greater than 1.0 μm between N and S cytoplasms. However, mtDNAs of S cytoplasm lack the minicircles within 0.28 and 0.4 μm size class, which are present in the N-mtDNA.
fraction. The results on the electrophoretic analysis of mitochondrial DNA (mtDNA) restriction fragment patterns have revealed distinctive characteristics; the N & S cytoplasms bear lightly stained tubular cristae (Fig. 6a-d). They degenerate abruptly and by the time microspores are released, from the tetrads and they grow into pollen grains and develop exine,

**Fig. 6 a-d.** Transmission electron microphotographs of parts of anthers of different CHAs treated plants showing abnormal tapetal mitochondria. a. Tapetal cell of anther of *Vicia faba* treated with benzotriazole showing increase in number of mitochondria 2400X. b. Degeneration of mitochondrial cristae in the tapetal cell of anther of *Vicia faba* plant treated with ethrel 2900X. c. Tapetal cell of anther of *Brassica juncea* treated with surf excel showing increase in number and size of mitochondria. 5400X d. Enlarged mitochondria with disintegrated cristae in tapetal cell of anther of ethrel treated *Cicer arietinum* 1450X.

possess characteristic sequences of mtDNA (Mikami et al., 1984; Chauhan, 1988).

The chloroplast DNA isolated from MF and CMS plants and subjected to restriction enzymes has failed to show differences except when subjected to Hind III digestion. In the latter case one band was missing from S chloroplast genome indicating that the involvement of ctDNA in CMS cannot be ruled out (Mikami et al., 1986; Chauhan, 1988).

The ultrastructural studies carried out by us on various chemically induced male sterile plants have clearly demonstrated degeneration of various cell organelles, mitochondria and chloroplast in particular in the malformed tapetal cells, almost similar to that recorded in CMS plants. Another interesting feature recorded in the tapetal cells of anthers of benzotriazole and ethrel treated plants of *Vicia faba* and surf excel- (a detergent) treated plants of *Brassica juncea* is significant increase in the number of mitochondria during early stages of development i.e. microspore tetrad stage. However, these mitochondria enlarge in size and the mitochondria degenerate almost fully. On the contrary, the mitochondria of the tapetal cells of anthers of plants treated with other chemical hybridizing agents remain small in size and their outer and inner (cristae) walls are significantly thickened (Fig. 6-d). These abnormalities in the behaviour of tapetal mitochondria of sterile plants seem to disturb respiratory metabolism of the anthers and lead to pollen abortion. Degeneration of cristae in the tapetal cells of the sterile anthers of chemically treated plants appears to be similar to that recorded in large number of CMS plants (Chauhan and Kinoshita, 1995). Molecular evaluation of mtDNA and ctDNA of such plants will be of great value for understanding the mechanism of pollen abortion in chemically induced male sterile plants. The tapetal cells also possess enlarged plastids with distorted lamellae. However, they are full of lipid and starch.

We have undertaken some experiments on sterile anthers of chemically treated plants to understand the mechanism of sterility on molecular basis. The RAPD analysis using three
primers on completely sterile anthers of *Vicia faba* plants treated with benzotriazole, ethrel and surf excel have indicated differences only in

Table 2: RAPD analysis of chemically induced male sterile *Vicia faba*

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>Total number of bands produced</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>OPH-07</td>
<td>5'CTGCATCGTG 3'</td>
<td>5</td>
</tr>
<tr>
<td>OPK-15</td>
<td>5'CTCCTGCCAA 3'</td>
<td>5</td>
</tr>
<tr>
<td>OPZ-06</td>
<td>5'GTGCCGTTCA 3'</td>
<td>8</td>
</tr>
</tbody>
</table>

compared to anthers of the male fertile plants. On the other hand *callase* gene is suppressed in the anthers of surf excel treated plants, and it is over expressed in the anthers of gibberellic acid, benzotriazole and ethrel treated plants (Chauhan and Singh, 2006). The suppression or over expression of these genes in sterile anthers leads to sterility. Molecular analysis of proteins is under way.

**CONCLUSION**

Tapetum is an important tissue of the anther, which disintegrates gradually with age and finally disappears but not before the formation of tiny grains carrying male genetic material. It also helps in development of endothecium, anther dehiscence and release of pollen grains from the microsporangia. Tapetum secretes the enzyme *callase*, which helps in the release of microspores from common callose wall. Precocious degeneration of callose leads in pollen abortion. Generational or induced disturbances lead to tapetal malfunctioning of various kinds, causes pollen abortion or male sterility, which is useful for hybrid seed production.
Molecular analysis in the anthers of male fertile and CHAs treated male sterile plants of *Brassica juncea*. 

**Fig. 8a-c.** Electrophoretic pattern of RAPD analysis with different oligonucleotide primers in the anthers of control and CHAs treated male sterile plants. **Fig. 8a.** RAPD analysis with primer OPH 07 in the anthers of plants treated with different CHAs. **Fig. 8b.** RAPD analysis with primer OPK 15 in the anthers of plants treated with different CHAs. **Fig. 8c.** RAPD analysis with primer OPZ 06 in the anthers of plants treated with different CHAs.

Where **M:** Marker Lane 1: Electrophoretic pattern of male fertile control plants. **Lane 2:** Electrophoretic pattern of surf excel treated plants. **Lane 3:** Electrophoretic pattern of benzotriazole treated plants. **Lane 4:** Electrophoretic pattern of ethrel treated plants. **Lane 5:** Electrophoretic pattern of gibberellic acid treated plants. **Fig. 8d-e.** DNA and RNA dot blot analysis in the anthers of male fertile and CHAs treated male sterile plants for anther specific genes *Bcp1* (A) and *Callase* (B). **Fig. 8d.** DNA dot blot analysis for anther specific genes *Bcp1* (A) and *Callase* (B). **Fig. 8e.** RNA dot blot analysis for anther specific genes *Bcp1* (A) and *Callase* (B).

Where, **1:** DNA and RNA of male fertile control plants. **2:** DNA and RNA of male sterile surf excel treated plants. **3:** DNA and RNA of male sterile benzotriazole treated plants. **4:** DNA and RNA of male sterile ethrel treated plants. **5:** DNA and RNA of male sterile gibberellic acid treated plants. **Fig. 8f.** RT-PCR analysis for anther specific gene *Bcp1*. Where, **M:** Marker **1:** RNA of male fertile control plants. **2:** RNA of male sterile surf excel treated plants. **3:** RNA of male sterile benzotriazole treated plants. **4:** RNA of male sterile ethrel treated plants. **5:** RNA of male sterile gibberellic acid treated plants.
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