Anther Ontogeny in an Exotic and Six Indigenous Cytoplasmic Male Sterile Lines of Wheat (*Triticum aestivum*) Possessing *Triticum timopheevi* Cytoplasm

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Anther ontogeny in an exotic cytoplasmic male-sterile (CMS) line 'Nadadores' and that in six indigenous CMS lines obtained through crosses with the former have been compared. In the exotic CMS line, pollen abortion was associated with nondegenerating and persistent tapetum. Similar abnormalities were recorded in the indigenous CMS lines also. Besides a few other tapetal abnormalities like degeneration of tapetum in premeiotic stages, tapetal hypertrophy in both pre and post-meiotic stages and pseudo-periplasmodium formation were also recorded. It is concluded on the basis of these observations that malfunctioning tapeta of various types are caused by inhibition of vascular tissue of the anther connective.

Introduction

The utilization of male sterility has progressed in the commercial production of hybrid seeds in self- and cross-pollinated crops (Duvick 1968, Heyne and Livers 1968). A number of cytoplasmic male sterile lines have been developed in wheat by Kihara (1951), Fukasawa (1953, 1959) and Wilson and Ross (1962) by the substitution of cytoplasmic background of *Aegilops caudata*, *Ae. ovata* and *Triticum timopheevi* in bread wheat genome respectively. The present paper is a part of the investigation carried out for transferring male-sterile character from an exotic CMS strain with *T. timopheevi* cytoplasm to some indigenous wheat varieties. The anther ontogeny in exotic as well as indigenous male-sterile lines has been worked out and compared.

Materials and Methods

Source of male-sterile cytoplasm and its transfer:

The cytoplasmic male-sterile (CMS) strain of hexaploid wheat 'Nadadores' possessing *Triticum timopheevi* cytoplasm obtained from Dr. Don L. Keim, spring wheat breeder, Cargill Research Farm, Colorado, U. S. A. was used in the present investigation. From this exotic male-sterile line pollinated by indigenous varieties, HD₁₅₉₃, HD₂₀₀₉, K₇₄₃₅, UP₃₆₈, WH₁₅₇ and WL₇₁₁, F₁ hybrids were obtained and by repeated backcrossings, stable male sterile lines were selected.

Spikes of male-sterile lines with their fertile counterparts at different stages of development were fixed in formalin-alcohol. For morphological studies these spikelets were dehydrated through tertial-butyle alcohol series and embedded in paraffin wax. Serial microtome sections, 10-15 microns in thickness were stained with Heidenhain's iron-alum haematoxylin and mounted in Euparal. Squashes of the anthers were made in 0.5%

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acetocarmine.

Observations

A. Anther ontogeny in fertile line:

Early in anther ontogeny, each microsporangium differentiates into four distinct tissues, epidermis, two parietal layers, a seriate tapetum and the sporogenous mass. The tapetum is similar in appearance and behaviour throughout microsporogenesis on both external and internal connective sides of the locules. Microsporogenesis has been divided into seven stages. The sporangial tissues are initially evident, the last stage ends near the anthesis after the tapetal protoplasts have disintegrated, and the pollen grains have become engorged with reserve food material. The changes that occur during anther ontogeny in male-fertile and male-sterile lines are presented separately, but within the framework of the seven stages.

(a) *Sporogenous mass stage*: This stage is marked by the complete cessation of cell division in both the sporogenous and tapetal tissues and by a considerable enlargement of their nuclei. Each tapetal cell is uninucleate and bounded by a primary wall. The chromatin of microsporocyte nuclei appears as a dense spherical body (Fig. 1). Differentiation of anther wall layers sets in showing larger tapetal cells and tangentially stretched middle layer cells (Fig. 2).

(b) *Meiosis I and II stage*: this stage starts with meiotic leptotene and ends with initiation of tetrad formation. Meiosis is normal and from squash preparations the chromosome number determined is $n=21$ at metaphase I and anaphase I. Meiotic prophase in PMCs is accompanied with highly synchronized karyokinesis in the tapetum and its cells become binucleate. Cells in both the tissues enlarge and occupy the space provided by the shrinkage of the middle layer (Fig. 3). The microspore mother cells, undergoing meiosis also form a hollow tube within the larger tube of tapetal tissue (Fig. 4).

(c) *Tetrad stage*: The enlargement of anthers leads to the separation of individual dyads and tetrads. The four meiotic products remain enclosed within a callose mother cell wall. The cells in middle layer and endothecium show further shrinkage and a loss of structural detail (Fig. 5).

(d) *Free microspore stage*: The release of young microspores from the callose wall marks the beginning of free microspore stage. This stage is transitory as each microspore soon undergoes rapid growth and it gives rise to uninucleate pollen. The cells of middle layer are disorganized completely.

(e) *Vacuolate microspore stage*: The young microspores rapidly increase in size and develop many vacuoles, which ultimately merge into a large central vacuole occupying almost the entire cell with only a thin cytoplasmic layer along the wall. This is accompanied by tapetal disintegration as is marked by their shrinkage and loss of protoplasmic contents.

(f) *Vacuolate pollen stage*: By the time the pollen grains reach vacuolate stage, the tapetal cells degenerate almost completely, leaving only a limited number of their narrow

Table 1. Enumerating the abnormalities observed during anther development in an exotic and six indigenous cytoplasmic male-sterile lines of wheat

Stage of development	Abnormality	Cytoplasmic male-sterile line					
		Nadadores	HD ₁₅₉₃	HD ₂₀₀₉	K ₇₄₃₅	UP ₃₆₈	WH ₁₅₇ WL ₇₁₁
Pre-meiotic	1. Degeneration of either sporogenous tissue, dyad or tetrad followed by degeneration of tapetum		+	+	+		+
	2. Degeneration of tapetum followed by degeneration of sporogenous tissue, dyad or tetrad		+	+	+		+
	3. Tapetal pseudoperiplasmodium development				+		
	4. Tapetal hypertrophy at sporogenous mass stage	+					+
Post-meiotic	5. Tapetum persisting up to anthesis	+	+	+	+	+	
	6. Undifferentiated tapetum				+	+	
	7. Tapetal hypertrophy at microspore stage	+					
	8. Tapetum degenerating at late vacuolate pollen stage and fibrous bands appearing on radial walls of endothelial cells			+	+		
	9. Pseudoperiplasmodium developing at pollen grain stage						+

protoplasmic fragments. Uni-, bi- or trinucleate pollen grains develop a thick exine. In this stage the characteristic fibrous thickening on the radial walls of endothelial cells starts to appear.

(g) *Mature pollen stage*: During the mature pollen stage the vacuole disappears and the pollen acquires dense, granular and dark stained cytoplasm filled with starch and other reserves. The fibrous bands in endothelial cells are fully developed at this stage while the tapetal cells are by now completely used up (Fig. 6). The mature pollen grains are possessing three nuclei, oval shaped with thick exine having a single circular germ pore.

In the anther, the procambial strands of the connective differentiate into well developed xylem elements enclosed by phloem tissue during meiosis stages I and II.

B. Anther ontogeny in male-sterile lines:

Ontogeny of anthers of exotic as well as indigenous male-sterile lines exhibits a number of abnormalities during pre-meiotic and post-meiotic stages, as summarized in Table 1.

1. Nadadores: Early ontogeny of anthers up to pre-meiotic stage, resembles that of fertile line. However, a number of abnormalities in subsequent stages are exhibited by wall layers, sporogenous tissue, microspores and anther connective.

The cells of epidermal layers elongate normally in tangential direction with their cuticle poorly developed. The endothelial cells fail to elongate radially with no fibrous bands on their radial walls. The middle layer in most of the anthers collapses at early microspore stage, only in a limited number of anthers do these cells persist up to anthesis.

The reduction in the number of anther lobes is a common feature of 'Nadadores' line. On account of this the anthers are found to be either bi- or tri-sporangiate. It

Figs. 1-6. Transverse sections of anthers of male-fertile lines. 210 X

Fig. 1. Early sporogenous tissue stage.

Fig. 2. Sporogenous tissue stage with all the wall layers.

Fig. 3. Late sporogenous tissue stage.

Fig. 4. Meiosis I and II stage.

Fig. 5. Tetrad stage.

Fig. 6. Mature pollen grain stage.

Figs. 7-12. CMS 'Nadadores' line.

- Fig. 7. Transverse section of a floret showing reduction in the number of anther lobes. 90 X
- Fig. 8. Transverse section of a bi-sporangiate anther showing tapetal hypertrophy at sporogenous tissue stage. 210 X
- Fig. 9. Transverse section of a bi-sporangiate anther with tapetum persisting at vacuolate pollen stage. 210 X
- Fig. 10. Bi-celled pollen grains with well developed exine having a germ pore. 240 X
- Fig. 11. Bi-celled pollen grains with well developed exine having a germ pore. 210 X
- Fig. 12. Transverse section of a tri-sporangiate anther showing tapetal hypertrophy during postmeiotic stage. 210 X

Figs. 13-18. Transverse sections of anthers of indigenous CMS lines.

Fig. 13. HD₁₅₉₃ showing degeneration of tapetum at early meiotic stage. 120 X

Fig. 14. HD₂₀₀₉ showing empty anther. 120 X

Fig. 15. K₇₄₃₅ showing pseudoperiplasmodium at PMCs stage. 210 X

Fig. 16. K₇₄₃₅ showing complete degeneration. 210 X

Fig. 17. UP₃₆₈ showing undifferentiated wall layers at sporogenous tissue stage. 120 X

Fig. 18. WL₇₁₁ showing pseudoperiplasmodium at pollen grain stage. 120 X

is interesting to note that the reduction in the sporangial number is usually shown by the central stamen which is affected first, followed by the lateral ones (Fig. 7).

The deviation of tapetum from normal course makes it necessary to emphasize these for the sake of clarity.

a. *Tapetal hypertrophy in pre-meiotic stages*: The tapetal cells are found to elongate radially and become hypertrophied at pre-meiotic stage of some flowers in a hundred of spikelets. Their sporogenous cells are thus crushed by this abnormal enlargement of tapetal cells even prior to the onset of meiosis (Fig. 8).

b. *Non-degeneration and persistent tapetum*: The tapetal cells remain intact up to anthesis in most of the flowers of the spike. The intact tapetal cells stretch tangentially and at mature pollen stage, possess deeply stained protoplast with degenerated nuclei. Such anthers contain non-viable pollen grains characterized by their shrivelled nature and lack of granular cytoplasm (Fig. 9). However, exine development is not much inhibited and a circular germ pore is invariably present (Fig. 10). The squashes of mature anthers showed that mostly only uninucleate pollen grains aborted but a limited number of binucleate and trinucleate pollen grains with scanty degenerated protoplast are also discernible (Fig. 11).

c. *Tapetal hypertrophy in post-meiotic stage*: In another limited number of flowers of some spikelets, intact tapetal cells enlarge in radial direction and become hypertrophied at early microspore stage. The tapetal cells occlude the anther sac and crush the microspores. Hypertrophied tapetal cells possess scanty cytoplasm with large vacuoles and degenerated nuclei (Fig. 12).

The anthers exhibiting the above mentioned types of abnormalities show inhibition of vascular differentiation. The vascular tissue may remain pro-cambial, while in the case of tapetal cells remaining intact, a large number of thin walled narrow xylem elements appear with degenerating phloem on its periphery (Fig. 9).

2. *HD₁₅₉₃*: In most of anthers of this male-sterile line, pollen abortion is seen associated with persistent tapetum similar to that of CMS 'Nadadores'. However, in a limited number of anthers either sporogenous tissue, pollen mother cells, dyads or tetrads degenerate causing complete sterility. This is followed by the degeneration of tapetum. In still other cases exhibited by a limited number of flowers, the tapetal cells degenerate at early meiotic stages (Fig. 13). This is followed by the degeneration of PMCs. Vascular tissues in either case remain undifferentiated.

3. *HD₂₀₀₉*: This male-sterile line, besides exhibiting intact tapetum associated with pollen abortion, also exhibits either complete degeneration of sporogenous tissue followed by tapetal degeneration or *vice-versa*. Such anthers are empty and are enclosed by epidermal layer alone. However, at places, endothelial cells are also seen (Fig. 14).

4. *K₇₄₃₅*: The male sterile line K₇₄₃₅, peculiarly enough, showing tapetal pseudoperiplasmodium at PMCs stage is of frequent occurrence (Fig. 15). The plasmodium degenerates along with the PMCs (Fig. 16). In other respects, it resembles in behaviour of its tapetum with CMS HD₂₀₀₉ line. In some cases, however, confined only to a limited

number of flowers, tapetum degenerates at the late vacuolate pollen grain stage. This is followed by the appearance of fibrous bands on the radial walls of endothelial cells.

5. *UP*₃₆₈: The development of male-sterile anthers of CMS *UP*₃₆₈ is similar to CMS *HD*₂₀₀₉. An interesting finding of tapetal cells in this line is that tapetum fails to give any impression of being a metabolic jacket at any stage as its cells possess poorly stained scanty protoplast (Fig. 17). In a limited number of anthers tapetal degeneration occurs at late vacuolate pollen stage followed by the formation of fibrous bands in the endothelial cells as shown by CMS *K*₇₄₃₅ line.

6. *WH*₁₅₇: This CMS line also exhibits degeneration of either sporogenous tissue, PMCs, dyads or tetrads as recorded in CMS *HD*₅₉₃ line. Poorly differentiated tapetum and tapetal hypertrophy in pre-meiotic stages are also of frequent occurrence apart from persistence of tapetum up to anthesis.

7. *WL*₇₁₁: Persistent tapetum similar to other CMS lines is a common feature. Besides, a limited number of anthers also exhibit tapetal degeneration at meiotic stages followed by the degeneration of PMCs. Tapetal pseudoperiplasmodium developing at pollen grains stage is of frequent occurrence (Fig. 18). The plasmodium degenerates along with the pollen grains.

Discussion

It is clear from the foregoing description of anther ontogeny in male-fertile and the CMS variety 'Nadadores' that abortion of pollen in this line is associated with nondegeneration and persistent tapetum. The tapetal cells in the anthers of CMS Nadadores persist throughout the course of development, rendering the microspores non-viable. Similar observations have also been recorded earlier in CMS wheat by CHAUHAN and SINGH (1966) and JOPPA *et al.* (1966). In the opinion of the present authors, the persistence of tapetum beyond certain stage seems to be deleterious for growth of microspores. The nondegeneration of tapetal cells may be responsible for not allowing the required nutrition to be transferred to the developing microspores which consequently abort. This interpretation is corroborated by the general appearance of tapetal cells and the vascular inhibition in such anthers. Further, poor staining capacity of tapetal cells in a limited number of anthers reflects their low metabolic state. Therefore, such malfunctioning tapetum, in the opinion of the present authors is neither capable of metabolizing anything nor capable of transferring nutrition to the developing microspores (see ECHLIN 1971). This hypothesis is further supported by the hypertrophying of tapetal cells in a limited number of CMS Nadadores anthers as suggested earlier by CHAUHAN and SINGH (1968) that the starving tapetal cells become haustorial in search of nutrition.

The formation of pseudoperiplasmodium in the anthers of CMS *K*₇₄₃₅ and *WL*₇₁₁ lines also seems to hinder the development of normal pollen grains, since their fertile counterparts exhibit only glandular tapetum. This is strongly supported by the observations of SINGH (1936) in *Ranunculus scleratus*. According to him, this plant shows glandular tapetum and rarely did periplasmodium develop, but whenever it was associated with

pollen abortion.

The anther ontogeny in the indigenous CMS lines developed through crosses with exotic variety 'Nadadores' also indicates similar mechanism of pollen abortion. However, a few other tapetal abnormalities in indigenous varieties have also been recorded as follows.

(i) Degeneration of tapetum followed by degeneration of sporogenous tissues or *vice-versa* has been recorded in CMS HD₁₅₉₃, CMS K₇₄₃₅, CMS HD₂₀₀, CMS WH₁₅₇ and CMS WL₇₁₁ lines. Recent ultrastructural studies have indicated the presence of plasmodesmatal bridges between sporogenous and tapetal cells during pre-meiotic stages (MASCARENHAS 1975). The premature breakdown of either tapetum or sporogenous cells breaks the communication between the two tissues and finally causes complete sterility.

(ii) In the anthers of CMS K₇₄₃₅ and CMS WL₇₁₁ lines, pseudoperiplasmodium develops at different stages of development, causing pollen abortion.

The above mentioned tapetal abnormalities, in the opinion of the present authors are caused by inhibition of vascular differentiation in the anther connectives. The occurrence of additional abnormalities in indigenous CMS lines may be due to the action of some other factors interacting with exotic cytoplasm of 'Nadadores'.

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Triticum timopheevi の細胞質で置換した7種のパンコムギ
雄性不稔系統における葯の發育異常

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パンコムギの近縁種 *Triticum timopheevi* の細胞質を置換して作成した雄性不稔系統 'Nadadores' (米国コロラド州カーギル研究農場産) を細胞質提供親として、インドの在来品種6種とそれぞれ交配し、さらに各在来品種を連続戻し交配して6種の雄性不稔系統を育成した。これらと原品種 'Nadadores' とを用いて、花粉退化に至るまでの葯の發育異常について比較観察を行った。

まず、原品種 'Nadadores' における葯の發育異常では、(1) 時々減数分裂期以前に、タペート細胞の肥大を生じ、花粉母細胞の退化を起こすことがある。(2) 大部分の小花で起こる現象として、開花に至るまでタペート組織が崩壊せずに残存する。そのため、小孢子の外皮の発達が抑制され、不完全花粉を生ずる。(3) 時には、小孢子形成初期にタペート細胞が放射状に肥大し、小孢子を圧迫して退化せしめる。一方、インド在来品種の核より成る6種の雄性不稔系統でも上記のようなタペート異常を生じたが、その外に、下記の如き異常を生じた。(1) 減数分裂期初期にタペート組織の崩壊に伴い、花粉母細胞や花粉四分子が崩壊する。(2) 減数分裂期以前または以後の時期に、タペート組織が周辺偽変形体 (Pseudo-periplasmodium) を形成し、小孢子の異常を起こす。

これらのタペート異常は、葯隔における維管束系の発達抑制と関連するものと考えられる。また、インド在来品種の核を有する雄性不稔系統において、原品種にみられなかった異常を新たに生じたが、これはおそらく *timopheevi* 細胞質とインド在来品種の核における遺伝要因間の相互作用に起因するものであろう。