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Morphological and Cyto-histological Expression of Male-sterility (*Triticum timopheevi* Cytoplasm) in Common Wheat

By

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With 4 figures

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Introduction

Cytoplasmic male-sterility is known to be induced when genomes of emmer and/or common wheat are substituted into the cytoplasms of *Aegilops boeoticum* Boiss., *Aegilops ovata* L., *Aegilops caudata* L., *Triticum monococcum* L., *Triticum zhukovskyi* Men. & E. R., *Triticum araraticum* Jakubz., *Triticum dicoccoides* (Körn.) Thell. var. *nudiglumis*, *Triticum timopheevi* Zhuk., *Triticum timonovum*, *Aegilops umbellulata* Zhuk., *Aegilops speltoides* Tausch, amphidiploid *Aegilops ventricosa—T. timopheevi*, amphidiploid *T. boeoticum—Aegilops squarrosa*, and *Secale cereale* L. (see Maan and Lucken 1972). Most of these cytoplasms, however, are associated with some undesirable side-effects. For instance, cytoplasm of *Ae. caudata* frequently produces germless grains, haploid and twin seedlings, and reduction in female fertility; while *Ae. ovata* cytoplasm causes delayed heading in male sterile lines compared to their counterfertile lines with normal cytoplasm (Kihara and Tsunewaki 1966). The most frequently used source of male sterility in hybrid wheat research derived from *T. timopheevi* apparently has no serious undesirable side effect; the expression of male sterility, although, has been found to be altered by the genetic background of recurrent parent (Sanchez-Monge 1968) and environmental conditions.

Anthers in most of the male sterile types develop to a certain stage but fail to produce functional pollen grains. The cytohistological pathway leading to pollen abortion investigated for *Ae. ovata*, *Ae. caudata* and *T. timopheevi* derived male sterility reveal that it varies (Fukasawa 1956, Chauhan and Singh 1966, Joppa et al. 1966) and may depend upon the source of cytoplasm. Fukasawa (1956) found that the behaviour of tapetum cells in male sterile

durum wheat (*Ae. caudata* cytoplasm) was apparently similar to that in self-fertile counter-part. While in male sterile hexaploid 'Norin' wheat (*Ae. ovata* cytoplasm), CHAUHAN and SINGH (1966) observed that the disorganized tapetum, tapetum plasmodium or delayed degeneration of tapetum cells was responsible for pollen abortion. The only study on microsporogenesis and anther development in male sterile hexaploid wheat having *T. timopheevi* cytoplasm by JOPPA, MCNEAL, and WELSH (1966) indicated poor differentiation of vascular bundles in stamens of male sterile anthers which resulted in reduced transport of solutes in anthers and subsequently abortion of pollen grains. According to these authors no other study of anther development in genetic or cytoplasmic male sterile plants has reported poor differentiation of vascular bundles in anthers of male sterile plants to be associated with pollen abortion. Because of this novel observation further cyto-histological observation on pollen abortion in male sterile hexaploid wheat having *T. timopheevi* cytoplasm was warranted.

The objective of the present investigation was to study the morphological and cyto-histological expression of *T. timopheevi* derived cytoplasmic male sterility in soft winter wheat (*Triticum aestivum* L. em. Thell.) with particular reference to the mechanism of pollen abortion.

Materials and Methods

A male sterile 'Bison' line (*T. timopheevi* cytoplasm) was obtained from the U.S.D.A. Crop Research Division, Beltsville, Maryland. Male sterility was transferred by successive back-crossings to several Ontario adapted soft winter wheat lines as mentioned in the results. The effectiveness of male sterility in each generation was checked by dehiscence of the anthers, and seed set under bagged ears.

Cyto-histological studies of the development of anther and pollen grains were conducted with Ms 48 — a male sterile line obtained after six back-crosses to 'Genesee', and the self-fertile line 'Genesee'. This male sterile (MS) line was completely sterile both in the field and in the growth room (STOSKOPF et al. 1970) under an 18 hour photoperiod and 25 ± .5 °C temperature during the light, and 18 ± .5 °C during the dark period. Florets were collected from male sterile and fertile plants grown in the field at Guelph (1968) that had anthers ranging from the archesporial cell stage to the mature pollen grain stage. The florets collected were fixed in Navashin's fixative and dehydrated in a TBA-ethyl alcohol series and embedded in paraffin (Melt. pt. 56.5 °C) according to standard procedure (JOHANSEN 1940). Transverse sections 6 μ thick were cut and stained in Delafield's hematoxylin or in crystal violet.

To study meiotic stages, temporary slides were prepared from the microsporocytes of male sterile and fertile plants collected in Carnoy's fixative (6 : 3 : 1) and stained in aceto-carmine.

Results

Morphological Expression of Male Sterility

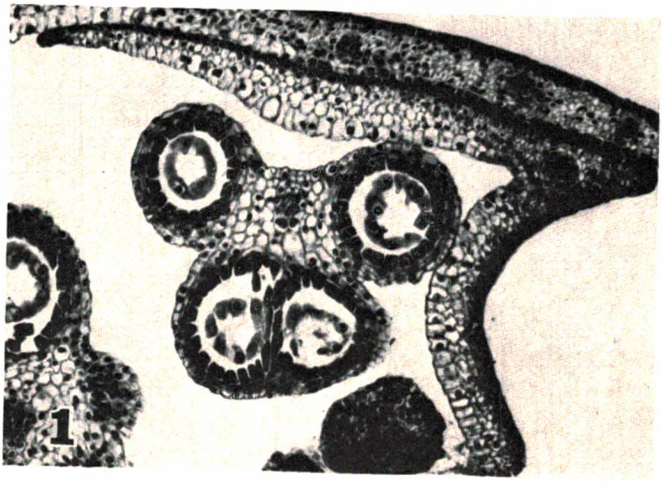
Differences in the expression of male sterility were observed during the transfer by back-crossing of male sterility to locally adapted soft winter wheat lines. Complete male sterility was obtained with 'Genesee', 'Hadden', 'Perdix', 'Fertödi', WW 3-1, WW 1-1, WW 3-4, 'Mironovskaya 808', 'Bezostaya', although WW 3-4, 'Fertödi' and 'Bezostaya' were partially fertile in first one or two back-cross generations. Partial fertility in F_1 and segregation for male

sterility was found when 'Timwin' was used as the male parent. The expression of male sterility was also influenced by the environmental conditions in which the plants were grown. Some male sterile lines, with the genetic back-ground of 'Bezostaya' and 'Fertödi' for instance, were more susceptible to environmental conditions compared to those lines with 'Genesee' in their back-ground.

The spikes of male sterile lines, before anthesis, appeared similar to those of the related fertile lines except for having malformed anthers. However, the male sterile florets remained wide open after anthesis until fertilized or the receptivity of the stigma was lost. Female fertility appeared to be unaffected. The kernels on male sterile spikes developed normally up to maturity but shrivelled slightly upon drying and contained a higher percentage of protein. Details on quality characteristics of kernel produced on male sterile plants are reported elsewhere by Rai et al. (1970). Variation in the shrivelling of kernels was noticed among the male-sterile lines ranging from very shrunken kernels, for instance of MS 'Genesee', to almost normal looking kernels of MS 'Bezostaya'. The shrivelled kernels were also more prone to sprouting in the ear under wet harvest conditions. Other plant characters such as plant height, days to heading and maturity appeared to be unaffected by the cytoplasm of *T. timopheevi.*

Cyto-Histological Observations

Cyto-histological observations on microsporogenesis and anther development were made from the archesporial cell stage to anthesis. The anthers of fertile lines "regularly" differentiated into tetralocular structures. In contrast, anthers in male sterile plants occasionally produced tri-locular anthers. Most of the tri-locular anthers existed at an early stage of differentiation of anther lobes (fig. A-3); however some later became tri-locular by the fusion of the two posterior loculi during the anther development (figs. A-1, A-2). Such fusion occurred prior to the formation of microspores and should not be confused with fusion of anther loculi during the maturation of microspores. Microsporogenesis



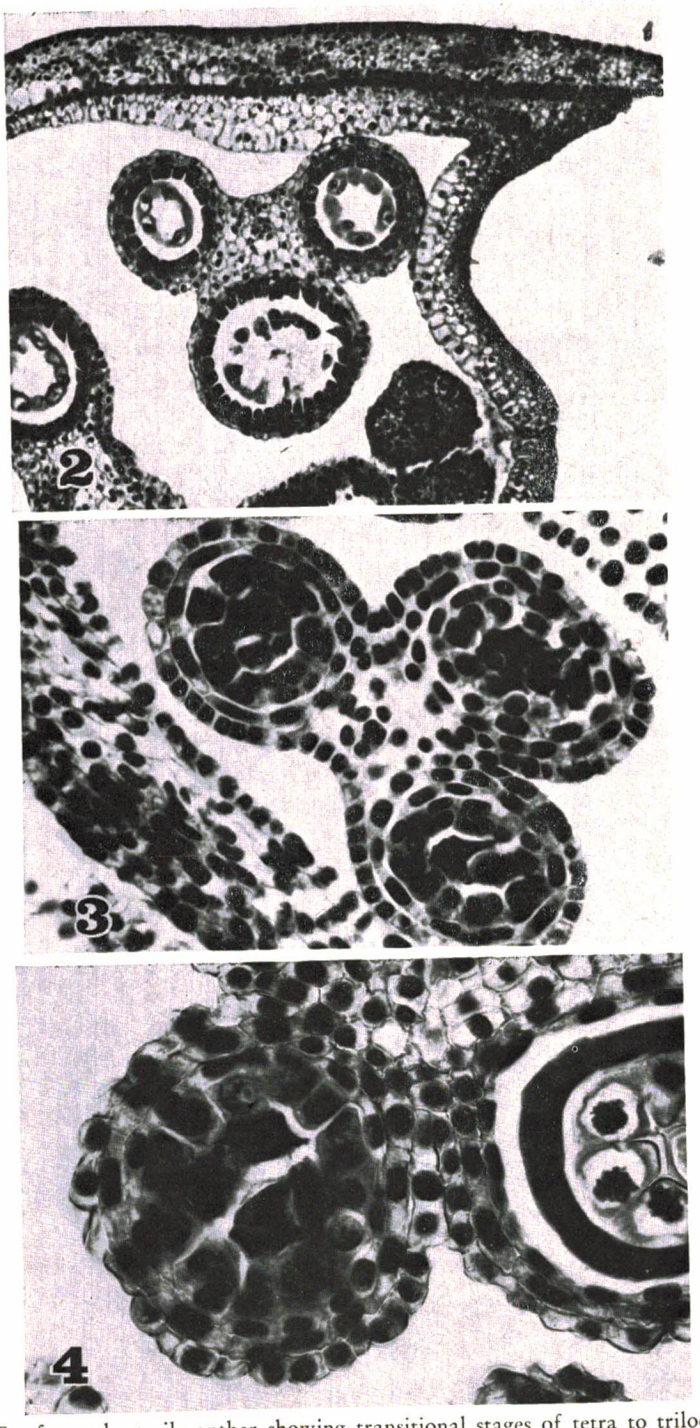


Fig. A. T.S. of a male-sterile anther showing transitional stages of tetra to trilocular anther × 210 (figs. A-1, A-2). Trilocular structure of a male sterile anther with degenerated meiocytes × 267 (fig. A-3). Note the hyper-trophied tapetal cells associated with degenerated meiocytes × 323 (fig. A-4)

in tri-locular anthers of male sterile lines did not appear to be different from microsporogenesis in tetralocular anthers.

In spite of differences in the number of anther locules of male sterile and fertile lines, constituent layers of anther wall such as epidermis, endothecium, middle layer and tapetum, were differentiated alike in all locules (fig. A-3) and were similar to those of the fertile line. Pollen mother cells (PMC) were formed in anthers of male sterile plants. Structural differences particularly in the tapetum cells of anthers of male sterile and fertile lines were encountered during (a) pre-meiotic, (b) meiotic, and (c) post-meiotic stages of anther development.

A) Pollen abortion during the pre-meiotic stage occurred after the formation of PMC's. The tapetum at this stage could be characterized by hypertrophic growth with large nuclei and abnormal vacuolation of tapetal cytoplasm (fig. A-4). The PMC's degenerated rapidly and did not advance beyond pro-

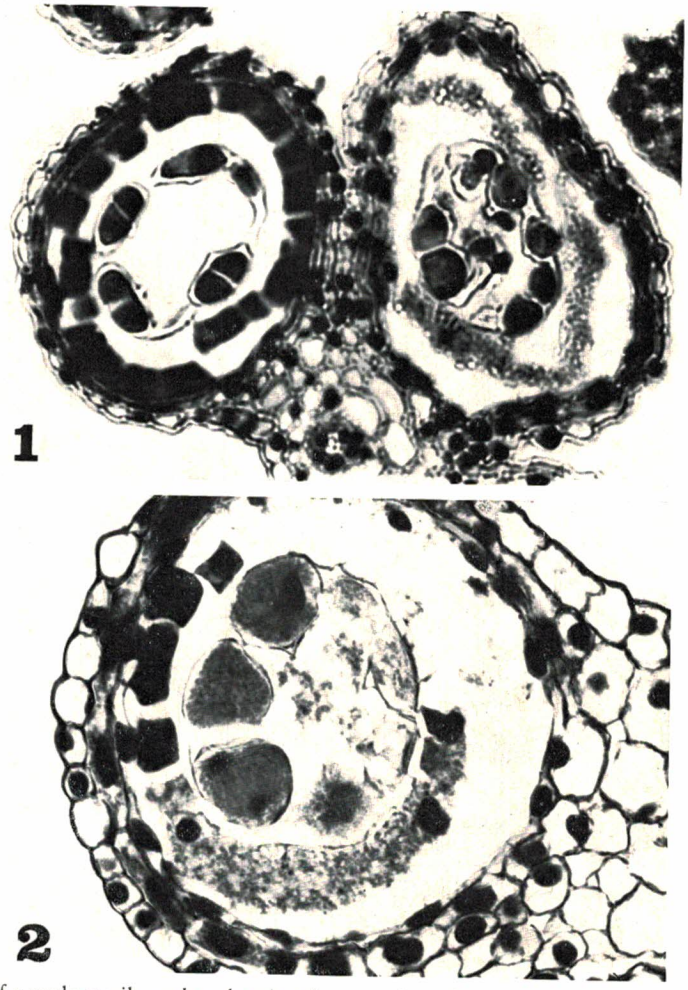
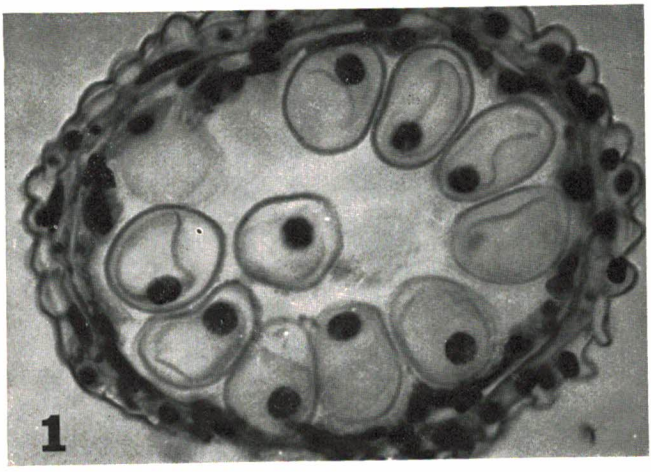


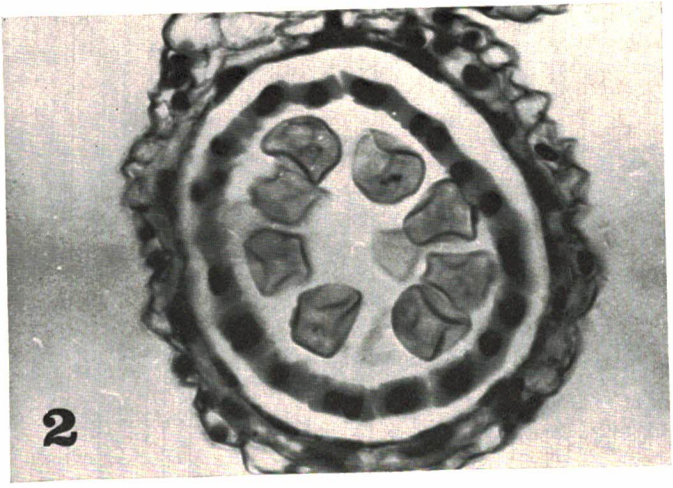
Fig. B. T.S. of a male-sterile anther showing degeneration of meiocytes at meiotic stages × 435 (fig. B-1), complete dissolution of tapetum before the degeneration of meiocytes is evident. Note the intact tapetum cells adjacent to non-degenerating meiocytes × 503 (fig. B-2)

phase of meiosis I. The tapetum cells later degenerated completely leaving the anther sac surrounded by epidermis and endothecial layers.

B) Aberrant behaviour of the tapetum was also observed during the meiotic division of PMC's. Tapetal degeneration in this case appeared to be brought about by sudden dissolution of its cell walls releasing the cytoplasm and nuclei into the anther locule (fig. B-1). The meiocytes, unlike the pre-meiotic stages, survived a little longer than the tapetum but did not develop after the degeneration of the tapetum. In events where a part of the tapetum layer in a locule remained intact the adjacent meiocytes did not degenerate (fig. B-2). Pollen abortion during pre-meiotic development of anthers occurred in low frequency and were sometimes limited to only posterior locule(s) of the anther.

C) In the majority of florets, pollen abortion took place during the post-meiotic development of anthers. Meiosis in PMC's and the development of tapetum in these florets appeared to be similar to that in fertile florets until the release of young microspores from tetrads. The tapetum in anthers of male sterile plants persisted longer (fig. C-2) as compared to that in anthers of fertile plants which gradually disappeared during the development of microspores





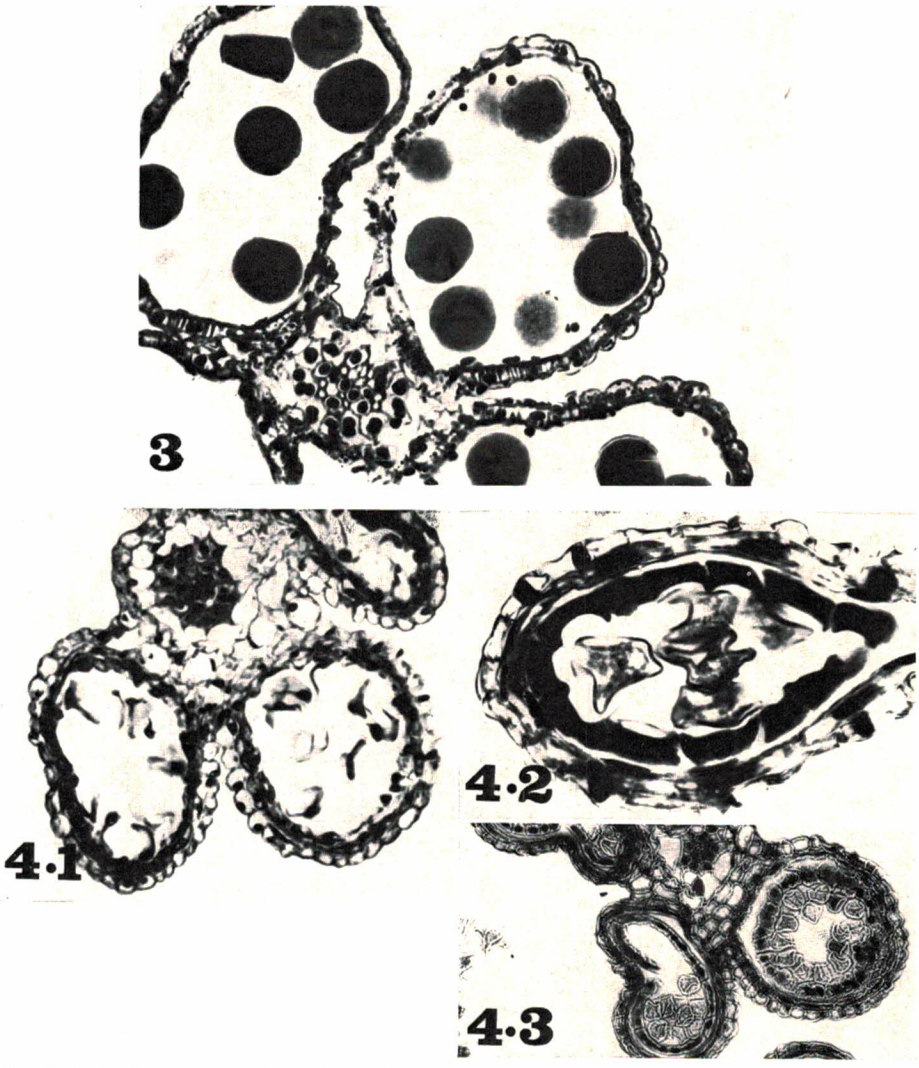


Fig. C. T.S. of a male sterile anther: Abortion of young microspores associated with cellular tapetum × 473 (fig. C-2). Fertile anther at the same stage of development × 450 (fig. C-1). Pollen abortion associated with partially absorbed tapetum × 231 (fig. C 4-1). Note the variation in tapetum's shapes associated with pollen abortion 458 × fig. C 4-2; 228 × fig. C 4-3; 225 × fig. C 4-1. Fertile anther at pollen grain stage × 214 (fig. C-3). Note the endothecial banding (fig. C-3) and lack of it in sterile anthers (fig. C 4-1)

(figs. C-1, C-3). At these stages the tapetum in anthers of sterile line could be represented by (i) cellular (fig. C 4-2) and/or (ii) plasmodial (fig. C 4-3) type of tapetum. The tapetum cells sometimes also appeared to be similar to those of the fertile one but only partially degenerated (fig. C 4-1). No endothecial banding was observed in mature anthers of the male sterile line in contrast to its conspicuous presence in fertile anthers (figs. C 4-1, C-3).

A diagrammatic summary of the main aberrant path-ways leading to pollen abortion in this study is presented in figure D. It is possible that sterility may

occur in other ways than those described in A, B, C. However, most of the observed aberrations can be fitted into one or other of these patterns.

Irrespective of the differences in behaviour of tapetum in the anthers of male sterile plants, pollen grains produced in each case were completely sterile. Microspores of sterile anthers had exine, intine and a conspicuous germ pore. They usually had one or two nuclei but rarely possessed three nuclei and were devoid of starch.

Apart from tapetal behaviour, the non-synchronous development of loculi in anthers of male sterile plants was also observed. In the event of degeneration of only one or two loculi at a particular stage of anther development, posterior loculi of anthers were found to be first affected (fig. B-1).

The vascular bundle system in filament and connective tissues of anthers of male sterile plants appeared to be well differentiated (fig. C 4-1).

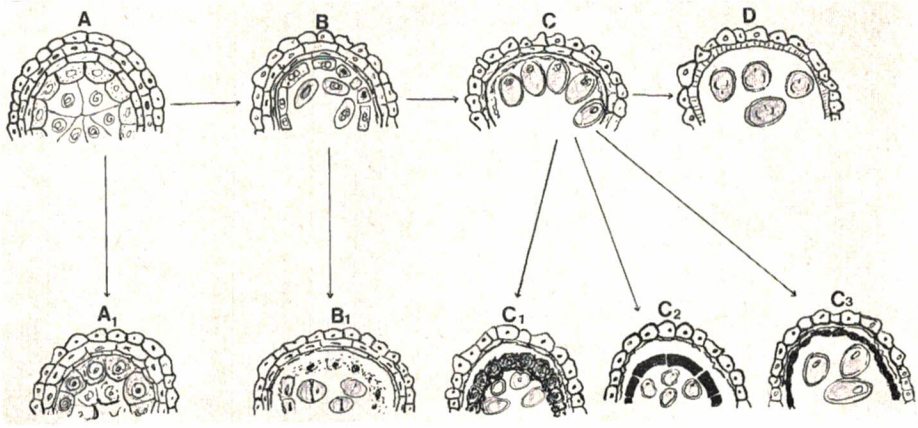


Fig. D. Diagrammatic summary of the main aberrant pathways. Top A to D: Normal sequence of anther development. A1, B1, C1, C2 and C3 refer to abnormalities at corresponding stages of anther development in male sterile

Discussion

As suggested by FUKASAWA (1962) male sterility probably results from a disharmonious relationship of the nucleo-plasmic system derived from two recognizably different sources. Therefore, the expression of male sterility in various normal lines of wheat might be expected to vary depending upon its genotype. The persistent partial fertility observed in some male sterile lines of this study may have been due to the presence of some nuclear factors for fertility restoration. Combination of such lines could possibly be used to produce complete fertility restorer lines if their fertility restoration factors behave in a cumulative and complementary fashion. Furthermore, the instability of male sterility in the genetic back-ground of certain normal lines probably represents a delicate equilibrium of a nucleoplasmic system which appears to be shifted easily under the influence of environmental conditions.

Morphologically, male sterile plants appeared to be similar to those of their self-fertile counter-parts. The open floret condition of male sterile plants during

anthesis aids in their easy identification as well as in hybrid seed production by cross-pollination. Though the female fertility of the male sterile plants appeared to be normal, shrunken seeds produced on these plants may have been due to a retarded synthesis of starch; as sterile pollen grains were also characterized by lack of starch grains. The sprouting of the shrivelled kernels in the ears may be associated with a high amylase activity in them.

Histological observations on development of anthers in male sterile lines indicated that an aberrant behaviour of the tapetum, similar to that of previous studies (ARTSCHWAGER 1947, CHAUHAN and SINGH 1966, WEBSTER and SINGH 1964), was associated with the abortion of pollen grains. The pollen abortion in this study during pre-meiotic, meiotic, and post-meiotic stages of pollen development in different florets of the same male sterile line was probably related to the position in a spikelet and/or spike; nevertheless controlled by a single mechanism of male sterility as this male-sterile line appeared to be homozygous. Pollen abortion in some florets during pre-meiotic and meiotic stages of pollen development may be considered characteristic of a "deep sterile" type line; as MS 48 was supposed to be a "deep sterile" type in our hybrid wheat programme.

Evidence from this study and those summarized by VASIL (1967) may suggest that the abnormality to the tapetum is bound to have an adverse effect on pollen development whether induced by genetic (RICK 1948), cytoplasmic (WEBSTER and SINGH 1964) or by environmental factors (KNOX and HESLOP-HARRISON 1966). However, the cause and effect relationship between the tapetum and pollen abortion is controversial (BROOKS 1966, WEBSTER and SINGH 1964) and difficult to establish because these two events occur simultaneously. Observations in this study suggest that the abnormality in the tapetum preceded pollen abortion. It does not, however, necessarily imply that all the causal factor(s) for male sterility originate in the tapetum cells. Biochemical studies (FUKASAWA 1968) indicate that metabolic abnormalities exist in male sterile plants at an early stage of plant growth. Abnormality in the tapetum then may be an intermediate but histologically distinct event associated with abortion of pollen grains by the virtue of its specific role (VASIL 1967) in the development of pollen grains. Differentiation of some anthers in male sterile plants into trilocular structures in this study, and in that of FUKASAWA (1956) may further support that an abnormality of some kind existed in male sterile plants before any noticeable change in the tapetum occurred. Sporophytic control of male sterility has been also indicated in several other cases (FUKASAWA 1955, JONES et al. 1957), the site of sterility producing factor(s), however, is unknown.

In the event of non-synchronous development of pollen grains within any one anther, the posterior loculi of the anther which were "relatively away" from vascular bundles degenerated first. This may indicate a "stress" in nutritional supply to the developing microspores in male sterile plants. Such a "stress" in the present study, however, did not appear to be caused by poor differentiation of vascular bundles in the anthers of male sterile plants as suggested by JOPPA et al. (1966). This study and that by TOWNLEY-SMITH (1968) indicated normal development of vascular bundles of male sterile plants. Incorporation of an equal amount of radio-active sugar and proline in anthers of fertile and sterile lines of wheat (ERICKSON 1967) may also provide an indirect evidence that the vascular bundles in anthers of male sterile lines were not poorly differentiated.

It may be suggested from this study that the tapetum is the main histological pathway controlling "events" leading to pollen abortion. The influence exerted by parts other than tapetum, however, also appear to be important.

Summary

1. Local lines of soft white winter wheat, when crossed with the male sterile line 'Bison' (*T. timopheevi* source), varied in their sterility expression ranging from complete to partial male sterility.

2. No serious adverse side effect of *T. timopheevi* cytoplasm in hexaploid male sterile lines of soft winter wheat was found. Female fertility was unaffected, although seeds produced on some male sterile lines were shrunken.

3. Cyto-histological observations indicated that pollen abortion could occur during (a) pre-meiotic, (b) meiotic, or (c) postmeiotic stages of pollen development with aberrant tapetum behaviour ranging from persistent to complete degeneration during pollen abortion.

4. The vascular bundles in anthers of male sterile plants developed normally. Reduced transport of solutes due to the poor vascular bundle development into anthers of the male sterile line did not appear to be a causal factor for pollen abortion.

5. Lack of synchronous development of pollen grains within any one anther, and tri-loculus anthers in male sterile line was observed occasionally. It was suggested that an abnormality of some kind may have been initiated at an early stage of micro-sporogenesis. The tapetum, however, may be a main pathway controlling "events" of pollen abortion.

Zusammenfassung

Morphologische und cytohistologische Manifestation männlicher Sterilität (*Triticum timopheevi*-Cytoplasma) bei Weizen

1. Lokale Linien eines weißen Weichwinterweizens variierten nach Kreuzung mit der männlich-sterilen Linie 'Bison' (*T. timopheevi*-Herkunft) zwischen völliger und teilweiser männlicher Sterilität.

2. Bei den hexaploiden männlich-sterilen Linien des Weichwinterweizens wurden keine wesentlichen ungünstigen Nebenwirkungen des *T. timopheevi*-Cytoplasmas gefunden. Die weibliche Fertilität blieb unbeeinflußt, obgleich die an den Pflanzen einiger männlich-steriler Linien angesetzten Körner geschrumpft waren.

3. Cytohistologische Beobachtungen ergaben, daß Pollenabortion während des prämeiotischen (a), meiotischen (b) oder postmeiotischen (c) Stadiums der Pollenentwicklung erfolgen kann. In allen Fällen wurde ein im einzelnen unterschiedliches aberrantes Verhalten des Tapetums während der Pollenabortion beobachtet.

4. Die Gefäßbündel in den Antheren der männlich-sterilen Pflanzen entwickelten sich normal. Ein reduzierter Transport von Lösungen in die Antheren der männlich-sterilen Linie scheint demzufolge als Ursache für die Pollenabortion auszuscheiden.

5. Gelegentlich verlief die Entwicklung der Pollenkörner innerhalb einer Anthere nicht synchron und in der männlich-sterilen Linie wurden auf einer frühen Stufe der Antherendifferenzierung Unregelmäßigkeiten beobachtet. Es wurde angenommen, daß eine gewisse Anormalität in einem frühen Stadium der Mikrosporogenese aufgetreten ist. Die zur Pollenabortion führenden Vorgänge dürften jedoch in erster Linie durch das Tapetum kontrolliert werden.

Acknowledgements

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