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ANATOMY AND CYTOLOGY OF MICROSPOROGENESIS IN CYTOPLASMIC MALE STERILE ANGIOSPERMS[1, 2]

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INTRODUCTION

Cytoplasmic male sterility (CMS) has been reviewed most recently by Edwardson (1970), who surveyed most of the literature through 1969. Nishi & Hiraoka (1958) and Chowdhury & Varghese (1968), not cited by Edwardson, have also reviewed the types and causes of pollen sterility in various crop plants, including CMS taxa. By our tally, there are published reports of CMS in approximately 140 species of 47 genera from 20 families of angiosperms.

We are interested in the anatomy and cytology of microsporogenesis[3] in normal (N) and CMS lines of the same species. While perusing research papers we tried to find out when exactly does abortion occur

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[2] Review of literature terminated April 1, 1972.

[3] This term, used here as by Horner & Lersten (1971), includes all stages from the undifferentiated microsporangium to the mature pollen-filled anther, including related events in the tapetum and external cell layers.

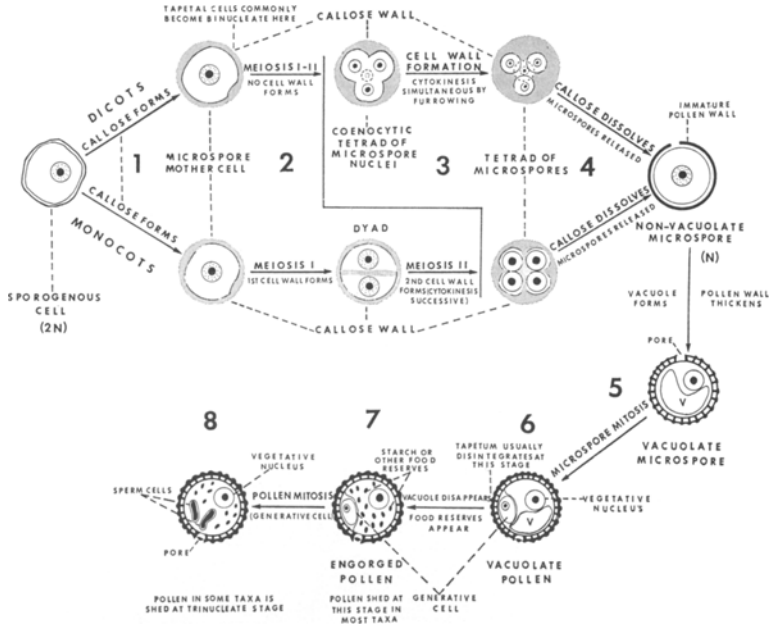

FIG. 1. A diagrammatic scheme of normal microsporogenesis in dicots and monocots. The large numbers 1–8 indicate stages explained in Table II.

and what happens when it begins, what clues can be observed before the onset of abortion and what related observable events occur in the tapetum and microspores during the stages leading up to abortion. We are impressed by the profusion and imprecision of much of the descriptive terminology, by the vagueness concerning answers to our questions, and by gaps in the descriptions of many authors. Some investigators seem unfamiliar with the morphological stages leading from sporogenous cells to mature pollen, as well as the terms used to describe these stages, or perhaps they simply have not considered that this aspect requires more than a passing general statement.

We also are impressed that abortion has been reported to occur during almost every stage of pollen development. This has stimulated us to study the literature comprehensively to determine as precisely as possible when morphological events occur, and to translate and reinterpret the heterogeneous descriptions into a homogeneous terminology that clearly and accurately describes these events so that they can be compared.

Existing reviews do mention when abortion occurs, as well as some of the accompanying morphological events, but not consistently for each species, nor always interpreted critically. With this in mind, we felt that a review stressing anatomical and cytological aspects would be a desirable supplement to those currently available and would not be redundant.

We also felt that including a diagram of normal microsporogenesis that was not too formidable in detail would aid investigators to understand this process better and, hopefully, help to stabilize the terminology. Such a scheme is presented in Fig. 1. We know that alternate terms exist for some that we have used and that certain details are omitted.

The anatomical and cytological events of microsporogenesis reported in the literature are summarized, with other pertinent items in tables for quick retrieval and comparison, because we feel that this information is least confusing when presented in such a form.

ORGANIZATION OF THE TABLES

There are four tables, which allow one to readily extract several kinds of information. Although the known literature has been covered comprehensively, we realize that some published accounts may have been missed.

Table I lists taxa in which CMS anther anatomy and cytology have been discussed and the investigators. Table II codifies three kinds of information into numbers for inclusion in Tables III and IV. Table III shows the distribution of reported stages of abortion. The vagueness of many reports, however, made it sometimes difficult to interpret abortive stages in terms of our eight stages of microsporogenesis. After the abortive stages were determined, they were tabulated as percentages of abortion.

Table IV contains our interpretation of the information taken from publications concerning anther morphology, microspore development until abortion, what occurs at the time of abortion, and changes in the tapetum. Investigators whose observations are similar are grouped together to avoid repetition. Clearly presented, pertinent information was translated into the terminology of Fig. 1. Where this information was difficult to interpret, it resulted in many rather cryptic phrases in the table. The phrase "no information" occurs frequently, emphasizing the incompleteness of many studies.

DISCUSSION BASED ON THE TABLES

TABLE I. This table lists 13 families, 26 genera, and 38 species. Monocots are represented by 2 families (Gramineae and Liliaceae), 9 genera, and 18 species. The grasses, as expected, have been studied most, with 8 genera, and 16 species listed. The dicots are a more varied group of 11 families, 17 genera, and 20 species. All taxa are listed as Latin binomials even though in many papers only a common name is used. Some reports omit reference to a species and are indicated as "sp." Investigators are listed with CMS species studied and assigned a number, which is listed in Table III.

TABLE II. This table includes sources of CMS, stages at which abortion occurs, and research techniques used. The CMS sources are those of Edwardson (1970): 1) from intergeneric crosses, 2) from

TABLE I. Cytoplasmic male sterile taxa that have been studied cytologically

Taxa Investigated	Investigator
Gramineae	
1.[a] *Aegilotricum*	
Amphidiploid of *Triticum durum* × *Aegilops ovata* cytoplasm	15, 16[b] Fukasawa (1953, 1956)
2. *Dactylis*	
D. glomerata	14 Filion & Christie (1966)
3. *Hordeum*	
Amphidiploids of *H. jubatum* × *H. compressum* & *H. bulbosum* × *H. vulgare*	45 Schooler (1967)
4. *Lolium*	
L. multiflorum	31 Nitsche (1971)
5. *Pennisetum*	
P. typhoides	47 Singh & Sharma (1963)
6. *Sorghum*	
hybrid of S. *vulgare* var sudanense	1 Alam & Sandal (1967)
S. vulgare	3 Brooks, Brooks, & Chien (1966)
	26 Maunder & Pickett (1959)
	29 Narkhede, Phadnis & Thombre (1968)
	46 Singh & Hadley (1961)
S. bicolor	25 Laser (Unpublished)
Sorghum sp.	40 Raj (1968)

[a] These genus identification numbers are used in the first column of Table IV.
[b] These investigator identification numbers are used in the second column of Table IV.

TABLE I (*continued*)

Taxa Investigated	Investigator
7. *Triticum*	
Triticum sp. with *Aegilops ovata* cytoplasm	5 Chauhan & Singh (1966)
T. aestivum	7 DeVries & Ie (1970)
T. aestivum × cytoplasm of *T. timopheevi*, *T. zhikonskyi* & *T. timonovum*	13 Fedorova & Nettevich (1969)
hybrid of *T. aestivum* & *T. timopheevi*	22 Joppa, McNeal & Walsh (1966)
Triticum sp.	43 Savchenko & Lastovych (1965)
	44 Savchenko (1967)
	56 Wilson (1968)
8. *Zea*	
Z. mays	4 Chang (1954)
	8 Diaconu (1965)
	17 Gabelman (1949)
	19 Herich (1965)
	21 Jones, Stinson & Khoo (1957)
	23 Khoo & Stinson (1957)
	38 Orel (1967)
	41 Rhoades (1933)
	42 Rogers & Edwardson (1952)
	50 Spasojevic (1966)
	54 Turbin et al. (1969)
Liliaceae	
9. *Allium*	
A. cepa	27 Monosmith (1928)
	51 Tatebe (1957)
	55 Virnich (1967)
A. fistulosum	30 Nishi & Hiraoka (1958)
Chenopodiaceae	
10. *Beta*	
B. vulgaris	2 Artschwager (1947)
	20a Hosokawa et al. (1954)
	28 Nagao & Kinoshita (1962)

TABLE I (*continued*)

Taxa Investigated	Investigator
Ranunculaceae	
11. *Aquilegia*	
hybrid of *A. vulgaris* × *A. crysantha*	48 Skalinska (1928)
hybrid of *A. flabellata* × *A. truncata*	49 Skalinska (1931)
Cruciferae	
12. *Brassica*	
B. oleracea	30 Nishi & Hiraoka (1958)
B. napus	57 Yamaguchi & Kanno (1963)
13. *Raphanus*	
R. sativus	30 Nishi & Hiraoka (1958) 37 Ogura (1968)
Rosaceae	
14. *Fragaria*	
hybrid of *F. vesca* × *F. nilgerensis*	34, 35 Nürnberg-Krüger (1956, 1958)
Leguminosae	
15. *Crotalaria*	
C. mucronata	11 Edwardson (1967)
16. *Lupinus*	
L. mutabilis	39 Pakendorf (1970)
Linaceae	
17. *Linum*	
L. usitatissimum	9 Dubey & Singh (1965)
Onagraceae	
18. *Epilobium*	
hybrid of *E. montanum* × *E. roseum*	36 Oberreuter (1925)

TABLE I (*continued*)

Taxa Investigated	Investigator
Umbelliferae	
19. *Daucus*	
D. carota	52 Thompson (1960) 58 Zenkteler (1962)
Apocynaceae	
20. *Tabernaemontana*	
T. coronaria	10 Dubey (1970)
Solanaceae	
21. *Solanum*	
hybrid of *S. chacoense* × *Solanum* sp.	18 Grun & Aubertin (1966)
Solanum sp.	24 Lamm (1941)
22. *Lycopersicon*	
Lycopersicon sp.	30 Nishi & Hiraoka (1958)
23. *Capsicum*	
C. annuum	32 Novak (1971) 33 Novak, Betlach & Dubovsky (1971)
24. *Nicotiana*	
hybrid of *N. tabacum* × *N. glutinosa*	53 Tsikova (1969)
25. *Petunia*	
hybrid of *Petunia* sp. × *P. hybrida*	12 Edwardson & Warmke (1967) 20b Izhar & Frankel (1971)
Cucurbitaceae	
26. *Cucumis*	
C. melo	6 Chauhan & Singh (1968)

TABLE II. Keys for interpretation of columns 4, 5, and 6 of Table IV.

Source of cytoplasmic male sterility (Edwardson, 1970)		Stages at which abortion may occur		Research technique used	
No.	Type	No.[a]	Stage	No.	Technique
1	arising from intergeneric crosses	1	sporogenous mass—microspore mother cell	1	standard paraffin or plastic sections
2	arising from interspecific crosses	2	meiosis I—meiosis II	2	squashes
3	arising from intraspecific crosses	3	tetrad	3	viability staining
4	arising apparently spontaneously	4	early non-vacuolate microspore	4	histochemistry
		5	vacuolate microspore	5	electron microscopy
		6	vacuolate pollen	6	scanning electron microscope
		7	engorged binucleate pollen	7	technique not mentioned
		8	tri-nucleate pollen		

[a] These numbered stages are illustrated in Fig. 1.

interspecific crosses, 3) from intraspecific crosses, and 4) apparently occurring spontaneously. This classification, which Edwardson said was arbitrarily assigned, seems reasonable and is convenient because most papers dealt with here also are included in his review. Among the taxa we have listed, 6 (10 per cent) came from source 1; 10 (16 per cent) from source 2; 11 (18 per cent) from source 3; and 35 (56 per cent) from source 4.

In column three of Table II, research techniques are listed and number coded: Fifty-seven per cent of the investigators reported using either standard paraffin sectioning or squash techniques, or both. Many studies report using chromosome fixatives, such as Carnoy's, for both cytological and anatomical preparations. Such fixing solutions have very harsh effects on tissues and are no doubt responsible for introducing preparation artifacts later misinterpreted. Staining for pollen viability with iodine seems to be used routinely and is often implied, but not specified, as a procedure. At least 16 per cent of the studies failed to mention the methods used to observe microsporogenesis and microspore abortion.

TABLE III. The time of abortion for each study reviewed was assigned to one or more of the eight developmental stages, as shown in this table and in Fig. 1. Although some investigators clearly described the abortive stage, others described them in a more general way. Abortion often was cited merely as occurring sometime before tetrad until the mature microspore or sometime during microspore maturation. This made it difficult to assign a specific time of abortion, and the percentages therefore reflect the total number of "possible" times abortion occurred

TABLE III. Percentage distribution of published reports of abortion among stages of microsporogenesis.

Stage[a]	1	2	3	4	5	6	7	8
All Taxa	6	9	25	32	13	13	2	0
Monocots	2	2	23	34	16	19	4	0
Dicots	10	17	28	30	10	5	0	0

[a] As described in Column 2 of Table II and illustrated in Fig. 1.

at a given stage in Table I. A single study, for example, may mention an abortive time that, in terms of our scheme, would have to be scored for two or three stages.

TABLE IV. This table provides a summary of the anatomical and cytological observations of CMS taxa. Of the 62 studies[4] 35 (57 per cent) were concerned with monocots and 27 (43 per cent) dealt with dicots. Thirty-one of the studies described gross anther morphology prior to, or at, anthesis. Eleven of these reported empty anthers with no pollen, 12 described the anthers as shriveled or shrunken in appearance, and 8 reported them as malformed, small, or showing other abnormalities related to structure or color.

Tapetal observations were included in 37 of the studies; 32 of these were reported as the secretory type and five were described as the plasmodial (periplasmodial) type (Davis, 1966). Of the 20 monocot species for which tapetal observations were included, only four reportedly had a tapetum that degenerated rapidly. Only 17 of the dicot studies presented information about the tapetum. Most species studied had a secretory tapetum that generally remained intact during microspore ontogeny or persisted until after microspore abortion, with little cytological evidence of hypertrophy or degradation. In those species reported to have a plasmodial tapetum, the process of microspore abortion generally seemed delayed.

Of the species reviewed, approximately 90 per cent had normal microspore ontogeny before the time of abortion, while the remaining 10 per cent showed abnormalities before abortion. The majority (about 70 per cent) of microspore abortions occur between stages 3 and 5. The proportion of abortions during these stages is quite similar for monocots and dicots. More dicots abort prior to tetrad formation (stage 3), but more monocots reach or persist beyond the binucleate pollen stage (stage 6).

Studies that we feel have given clear (although sometimes limited) descriptions of cytological events of microsporogenesis and related tapetal behavior in CMS lines deserve mention. Some of these are: *Sorghum* (Singh & Hadley, 1961; Brooks, Brooks & Chien, 1966), *Zea* (Chang,

[4] "Studies" as used here represents 59 published papers; since the work of Nishi & Hiraoka (1958) included four different taxa, it was considered to represent four different studies, making a total of 62 studies.

TABLE IV. Summary of cytological observations on CMS taxa

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
1	15	+	1	3–4	2	Empty Pollen	Show hypertrophy, 3–4 nuclei present that degenerate	Microspores degenerate after tetrad stage	No information
	16	+	1	6	1, 2	13.3% of anthers malformed	Uninucleate or enucleate; some show normal mitosis	Pollen degenerates at microspore mitosis	No hypertrophy, no plasmodium formed, cells appear stretched tangentially
2	14	+	4	3–4	1, 2	Dark green, small, no pollen	Degenerate rapidly	Microspores degenerate after tetrad stage	Degenerates rapidly with cytoplasm loss. Remnants visible at anthesis
3	45	+	2	6–7	1, 2	No information	Normal development until abortion occurs	Development to nonviable pollen	Remains "thickened"
4	31	–	4	3–4	2, 3	No information	Normal development until abortion occurs	Microspores degenerate after normal meiosis	Remains intact
5	47	+	4	4	1, 2	No information	Normal development until abortion occurs	Abnormal development at early microspore stage	Remains intact

[a] See Table II for explanation of these numbers.
[b] See Table I for explanation of these numbers.
[c] See Table II and Fig. 1 for explanation of these numbers.

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
6	1	+	3	3–4	1, 2	Normal during premeiotic stages	Normal development until abortion occurs	Associated with non degenerating tapetum	Does not degenerate after meiosis; remains intact
	3	+	3	4–5	1	No information	Normal development, but no microspore mitosis or starch formation	Aborted microspores have normal wall development and germination pore	Variation in width and morphology
	25	–	3	4–5	1, 2, 3, 4, 5, 6	Short and shriveled in later stages	Normal development to mid or late vacuolate stage	Collapsed, vacuolate, cytoplasm appressed to wall; normal wall development	Remains intact until microspores are completely aborted
	26	+	3	5–6	3	Shriveled	Normal development until abortion occurs	Pollen grains partly formed	No information
	29	–	3	4–5	1, 2	No information	Degeneration before or after microspore mitosis	No information	Appears shriveled and degenerates before microspore mitosis
	40	–	3	1–4	1, 2	No information	Degenerate before microspore mitosis	Microspores not released from tetrad	Periplasmodium formed after meiosis I; reduction of cytoplasm and nuclei

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
	46	+	3	5–6	1, 2	No information	Develop intine and reticulate exine	Most abort as microspores, some as pollen	Multinucleate tapetum persists after abortion
7	5	+	1	3–4	1, 2, 3	No information	Normal development until degeneration	Degenerate at tetrad separation; microspore devoid of cytoplasm, nuclei and germ pore	Abnormal behavior in each floret. Persists after microspore degeneration
	7	–	3	6	5	No information	Normal development through exine formation	Lobed microspores with normal exine and thin intine; devoid of starch	CMS tapetum identical to tapetum of normal anthers
	13	–	1	6	7	No information	Cytoplasm degenerates	Cessation of microspore growth between microspore mitosis and pollen mitosis	No information
	22	–	1	6	1, 2, 3	Sickle shaped	Do not usually go through microspore mitosis	Microspores devoid of starch	Persists after abortion
	43, 44	–	3	3–4	7	Undeveloped	Contents degenerate	Cessation of microspore growth after meiosis	No information
	56	+	1	6–7	—	No information	Meiosis and microspore mitosis are normal	Pollen development is rarely completed	No information

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
8	4	–	4	4–5	1, 2	No information	Cytoplasm vacuolate and microspores degenerate after tetrad release. Microspores develop normal wall and germ pore.	Growth ceases after uninucleate microspore	Endomitotic and thickened. Becomes dissociated from parietal cells and free cells degenerate later than normal tapetum. Persists after abortion, degenerates rapidly
	8	–	4	5–6	1, 2	No information	Highly vacuolated during maturation	Degeneration at 1–2 nuclei stage	Remains intact
	17	+	4	4	3	No information	Abortion occurs during microspore development	No information	No information
	19	+	4	3–4	7	No information	Cytoplasm vacuolate, small transparent bodies without starch	First signs of degeneration after release from tetrad	No information
	21	+	4	4–5	1, 2	No information	Sterile microspore smaller with less cytoplasm than normal. 2–6 nucleoli of various sizes and cause of abortion	First signs of degeneration after release from tetrad	No information

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
	23	–	4	6	1, 2	Small, papery	After initial enlargement, development arrested. Wall thickening limited, faint pore develops, cytoplasm coarsely granular, no starch.	Degeneration at microspore mitosis	No information
	38	–	4	3–4	2, 3	No information	Degenerate after tetrad release in M-type CMS. In T-type CMS, degeneration during later microspore development	Abnormal development after release from tetrads	No information
	41	+	4	5	2	No information	Microspore walls collapse after cytoplasm degenerates	Abortion occurs before microspore mitosis	No information
	42	–	4	5–6	2	Anthers shrunken	Failure of starch production during microsporogenesis	Some binucleate pollen	No information
	50	–	4	3–4	2	Anthers empty	Egg-shaped, cytoplasm shrinks around nucleus	Light stickiness of chromosomes at abortion	No information

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
	54	–	4	6	2	No information	Large numbers of dyads, slower development of microspores	Development ceases after pollen becomes binucleate	No information
9	27, 51	+	4	2–3	2	No information	Normal development followed by degeneration	No information	Hypertrophy before abortion, abnormal degeneration
	30	–	4	3–4	1, 2	Anthers reduced, no dehiscence	Normal development until immediately after tetrad formation	Associated with enlarged, non degenerating tapetum	Enlarge immediately after release of microspores from tetrad. Vacuolated and appress the microspores. May be plasmodial
	55	+	4	3–4	1, 2	No information	Normal development until tetrad formation	No information	Remains intact until microspore contents are reabsorbed
10	2, 20, 28	+	4	1–3	1	Anthers empty	Normal development normal until release from tetrad	Some MMC begin to degenerate before meiosis is complete	Plasmodial or secretory tapetum is formed. Progressive degeneration of nuclei and cytoplasm. Plasmodial presence delays abortion

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
11	48, 49	+	2	6	7	No information	Normal development to pollen mitosis	Pollen develops generative nucleus and degenerates	No information
12	30	–	4	3–4	7	Reduced, no dehiscence	Normal development until release from tetrad	Associated with non degenerating tapetum	Remains intact
	57	+	4	2–3	7	No information	Reduction in MMC and pollen produced	No information	No information
13	30	–	4	3–4	7	Reduced, no dehiscence	Normal development until release from tetrad. Poor exine development.	Associated with non degenerating tapetum	Vacuolated, enlarged and appress microspores
13	37	+	4	4	4	Shrunken, papery	Normal development until release from tetrad	No information	Collapses as microspores degenerate. Early breakdown may induce microspore degeneration
14	34, 35	+	2	3–4	2	Shrunken	Normal development until release from tetrad	No information	No information
15	11	+	4	3–4	7	No information	Normal development normal until after meiosis	No information	Abnormalities frequently encountered

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
16	39	–	4	3–4	1, 2	Shriveled and darkened. Endothecium enlarges at abortion	Normal development through tetrad	Microspores fail to separate at tetrad	Remains intact
17	9	+	3	4–5	1, 2	No information	Normal development through the later microspore stages	Normal wall development, but do not become binucleate	Persists after abortion of microspores, remains cellular until degeneration.
18	36	+	2	2–3	7	No information	Normal development until the tetrad stage	No information	Normal development until microspore degeneration
19	52, 58	+	4	3–4	1, 2	Shrunken, brown	Normal development until tetrad release	Shrunken cytoplasm, pycnotic	Plasmodial complex is formed
20	10	–	4	4	1, 2	No information	Normal development until abortion	Associated with non degenerating tapetum	Persists and is the cause of abortion
21	18, 24	+	2	4–5	2	Empty	Normal development to dyad stage but not later than early exine	Normal wall with thick exine develops, but microspores become shriveled.	No information

TABLE IV (*continued*)

Genus[b]	Investigator[b]	Reviewed by Edwardson (1970)	Source of cytoplasmic male sterility[a]	Abortive stage[c]	Research technique used[a]	Anther Morphology	Observations of microspore development	Cytological observations at time of abortion	Observations of tapetal development
22	30	–	4	3–4	1, 2	Anther reduced, no dehiscence	Normal development until abortion	Associated with non degenerating tapetum	Remains intact after microspore abortion
23	32, 33	–	4	1–2	1, 2	Mature anthers contain no pollen	Degenerate at the end of telophase II	[d] Irregular meiosis of MMC	Hypertrophy begins during premeiosis, delayed degeneration
24	53	–	2	4–5	1, 2	No information	Normal development until late microspore stage	No information	Remains intact and large after abortion
25	12	+	2	2	3	Contain no viable pollen	No evidence of development after tetrad stage	No information	No information
	20b	–	2	2–3	1, 2, 4	No information	Development terminated after abortive stage	Cell wall is seldom laid down after Meiosis II	Begins degeneration at prophase I
26	6	–	4	3–4	1, 2, 4	Anther reduced, no dehiscence	Normal development until release from tetrad. Wall not differentiated into intine and exine	Microspores at tetrad fail to separate and remain cemented together, irregular masses	Cells invade locule at abortion and disorganizes the microspores.

[d] Study included because of reference to cytoplasmic male sterility.

1954), *Triticum* (Chauhan & Singh, 1966; DeVries & Ie, 1970), *Beta* (Artschwager, 1947), *Daucus* (Zenkteler, 1962), and *Cucumis* (Chauhan & Singh, 1968).

Artschwager (1947), with *Beta vulgaris* and Zenkteler (1962), with *Daucus carota* observed a plasmodial tapetum and commented on its role in delaying microspore degeneration. In sterile lines of *Beta*, pollen abortion is associated with either a periplasmodium (plasmodial) or a secretory tapetum; each tapetal type is associated with different sterility behavior. Both kinds of tapetum may occur within a flower cluster, but not within a single flower. The presence of a plasmodial tapetum somewhat delays pollen abortion, but where there is a secretory tapetum, the tapetal cells enlarge toward the locule center, appressing the microspore tetrads and causing abortion sooner than in the plasmodial type. The microspores degenerate at the tetrad stage, sometimes even beginning late in meiosis. The plasmodial tapetum becomes separated from the parietal layer, surrounds and separates the tetrads, and after callose dissolution, the microspores begin to degenerate. The plasmodial tapetum, with its associated delayed microspore abortion, also was observed in *Daucus*. Both authors concluded that the progressive degeneration of nuclei and cytoplasm in the plasmodial tapetum is identical to that observed in the tapetum of normal plants. They found it difficult to ascribe to the plasmodial tapetum any harmful influence on the developing and ultimately aborting microspores, except a noticeable delay in the abortion process.

The extensive studies of the tapetum in fertile, restorer, and sterile lines of *Sorghum vulgare* by Brooks, Brooks & Chien (1966) described a radially enlarged tapetum in sterile anthers associated with microspore abortion. In five fertile lines, in contrast, the tapetum decreased in radial extent after meiosis I. At the engorged pollen stage, 78 per cent of the fertile anthers and 88 per cent of the restorer anthers had a narrow tapetum measuring 4–16 μm wide. In the sterile lines, an enlargement of the tapetum occurred at the vacuolate microspore stage, the time of abortion. At this point, 68 per cent of the sterile anthers had a tapetum that measured less than 16 μm wide. The remaining

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This paper was overlooked during our literature survey: Damon, E. G. 1961. Studies of the occurrence of multiploid sporocytes in three varieties of cytoplasmic male-sterile and the normal fertile variety, resistant wheatland sorghum. Phyton **17**: 193–203. Acetocarmine squashes and paraffin sections were used to study five CMS sorghum varieties: Wheatland, Westland, Martin, Redlan and combine Kafir 60. The first three are closely related and Damon reports that in these lines primary wall and callose destruction occurs in the MMCs, thereby interfering with meiosis, resulting in aneuploid and multiploid dyads and microspores which abort without completing meiosis, or shortly after. In the latter two varieties early wall and callose degeneration was not observed, and development continued seemingly in normal fashion until some unspecified microspore stage (Damon uses the terms "young pollen" and "older pollen material," but from his text description it seems unlikely that microspore mitosis occurs).

32 per cent had a well developed or enlarged tapetum measuring over 28 μm in width. The tapetum of the sterile anthers showed a marked variation in width and morphology that was not observed in fertile lines. They associated the enlarged tapetum of the sterile anthers with degeneration of the microspores.

Chauhan & Singh (1968), working with *Cucumis melo* pollen abortion, reported an increase in acid phosphatase activity in normal anthers until the tetrad stage, after which this activity decreased and the tapetum degenerated sometime later in microspore development. In sterile anthers, acid phosphatase activity was always low, and the tapetum persisted, swelled, and finally crushed the microspores. The tapetal nuclei of normal anthers stained intensively with the Feulgen reaction until the microspore mother cell stage, after which staining became progressively weaker until tapetal collapse. In sterile anthers, the Feulgen reaction was intense even after tetrads formed, only decreasing later in microspore development.

These observations form the basis for their theory of tapetal function and its role in abortion. In normal anthers, the tapetum receives food material of some kind from elsewhere in the plant and transfers it to the young microspores. It also passes Feulgen-positive substances to the microspore mother cells at about the time of meiosis. In sterile anthers, the tapetum has a low level of metabolic activity, as indicated by low acid phosphatase levels, and it fails to pass the Feulgen-positive material to the sporogenous cells at the critical time. The novel aspect of their theory is the presumption that tapetal cells receive very little food from elsewhere and, as a consequence, "take to haustorial activity in search of nutrition." This predatory activity is directed toward the microspores, which collapse as a result.

They show that tapetal swelling occurs in *Cucumis* sterile anthers, but their histochemical evidence must be accepted with reservation, partly because they did not reveal their method. A very careful study by Moss (1967) and Moss & Heslop-Harrison (1967), using sophisticated quantitative microdensitometry, failed to clearly show that anything is transferred from the tapetum to developing microspores in *Zea mays*.

INFORMATION NOT IN THE TABLES

There are certain other aspects of CMS that should be discussed, but the works dealing with them are either unpublished or did not seem to fit well in the tables as we conceived them. They therefore are dealt with separately in the following paragraphs.

Palmer and Albertson (personal communication) at the Department of Agronomy, Iowa State University, are investigating CMS in a variety of *Glycine max*. They have shown that after normal meiosis, cytokinesis fails to occur and the resulting coenocytic tetrads each develop a typical pollen wall, after which they abort. The expected ratio of pollen grains in N anthers to sterile pollen grains in CMS anthers in this system therefore

is 4:1. Repeated counts of both paraffin serial sections and squash preparations of mature N and CMS anthers revealed instead a ratio of 2:1 normal to sterile pollen. A unique explanation was found when they observed approximately twice as many CMS microspore mother cells (MMCs) as normal MMCs in sections of very young anthers. Meiosis and failure of cytokinesis in the CMS anthers would naturally result in the 2:1 ratio. This is the earliest stage known at which there is an anatomical distinction between N and CMS anthers.

Palmer (1971) recently described another variation. In certain types of *Zea mays* the cells of the sporogenous mass undergo a synchronous mitosis instead of meiosis, after which they abort.

Absence of cytokinesis during microsporogenesis has been reported only once, in male sterile, amphidiploid *Aegilotricum* (Fukasawa, 1953). Microspore abortion occurs immediately after the tetrad stage, and the degenerating microspores have 3–4 nuclei present. Failure of cytokinesis may be more common, however, and in future CMS cytological studies, this stage of microspore ontogeny should be scrutinized more carefully.

There are numerous proposed causes of CMS in angiosperms, including induction by chemicals, irradiation and environmental influences. The literature in this area is too voluminous to allow an extensive review here; some representative studies that included anther cytology, however, will be mentioned.

Erichsen & Ross (1963) studied microsporogenesis in colchicine-induced, male-sterile mutants and normal lines of *Sorghum vulgare*. The PMCs of fertile anthers undergo normal meiosis, but abnormal PMCs in sterile anthers are joined to the tapetal cells by numerous cytoplasmic strands. These strands are thought to influence nutrient movement from the tapetum to the PMC, resulting in the failure of MMC primary wall degeneration during meiotic prophase. The tapetum of sterile plants persists longer than in normal plants. They concluded that mutant tapetal behavior is similar to that in regular CMS lines and that the induced mutation for sterility has a mechanism similar to that commonly used in the production of hybrid sorghum.

Kaul & Singh (1967), investigating *Allium cepa* and Dubey & Singh (1969), investigating *Coriandrum sativum* studied pollen abortion in chemically induced male-sterile lines. The test plants were treated with varying concentrations of sodium 2, 3-dichloroisobutyrate (Mendok) and maleic hydrazide (MH). None of the treatments with Mendok caused complete pollen sterility, but concentrations of MH at and above 0.01 per cent caused 100 per cent sterility in both species. MH also produced abnormal tapetal behavior in *Coriandrum*. In untreated N and CMS lines of *Coriandrum*, the tapetum degenerated after meiosis, and abortion occurred shortly after microspore release from the tetrad. In MH-treated *Coriandrum* plants, the tapetum elongated and persisted until the vacuolate microspore stage. It was thought that, in *Allium*, MH interfered with pollen formation by causing premature degeneration of

sporogenous tissue or MMCs, and that in *Coriandrum*, microspore abortion of induced male steriles was attributed to "lack of change of nuclear size and unusual elongation of cells of the tapetum . . . ."

Kinoshita & Takahashi (1966) and Kinoshita & Nagao (1968) investigated pollen sterility in *Beta vulgaris* induced by gamma irradiation. They obtained complete as well as partly male-sterile plants from irradiated seeds (dosages of 50–200 kr), with sterility maintained in the first generation after irradiation. Tapetal abnormalities were always associated with pollen abortion. They suggested that male sterility is controlled by a mutation of a cytoplasmic factor, converting normal into sterile cytoplasm.

There have been numerous studies attempting to correlate the influence of various environmental factors with the endogenous factors thought to regulate anther development, thereby inducing male sterility. In an early study on *Sorghum*, Stephens (1937) observed subtle influences of light and temperature in producing withered, sterile anthers in otherwise normal flowers, resulting in changes in the time of anthesis and pollination. Heslop-Harrison (1957) has reviewed extensively work done previously on the experimental modification of sex expression, including male sterility, in angiosperms. Peterson (1958) noted that in CMS *Capsicum*, sterility is accentuated at temperatures above that of normal seasonal growth. On the other hand, Kidd (1961) observed that an increase in male fertility in CMS plants of field sorghum containing milo cytoplasm was associated with high temperatures. Low temperatures (daily highs of 50° F or less) during flowering markedly reduced pollen fertility.

Certain environmental conditions seem to act on some internal system by either promoting or inhibiting an auxin-like substance that, in turn, influences pollen formation and breakdown of the tapetum. Heslop-Harrison & Heslop-Harrison (1958) made cytological observations of *Silene pendula* anthers with long-day and auxin-induced sterility, noting that failure to produce normal pollen resulted from early tapetum degeneration with subsequent failure of normal transfer of materials to the microspore. During normal pollen development, the tapetum usually enlarges, pollen becomes engorged, and the tapetum subsequently degenerates, and in sterile pollen, there is an inverse correlation between density of pollen contents and the bulk of tapetal residue. Under long-day conditions, most microspores become fully enlarged and highly vacuolate, with the nucleus appressed to the wall; the tapetal cells form a densely staining sheath around the locule. Some microspores abort prior to enlargement, but most abort at anthesis. The aberrations of microsporogenesis resulting from long-day exposure for several days seem to arise from early degeneration of the tapetum. These effects can be simulated by auxin treatment. Abnormally high concentrations of auxin were noted in plants exposed to long photoperiods. This supports the

view that the influence of photoperiod on sex expression is exerted through native auxin metabolism in the plant.

The influence of photoperiod on pollen sterility in *Zea mays* was shown by Moss & Heslop-Harrison (1968); all floral abnormalities produced by plants grown in 8-hour days could be negated by a night interruption at low light intensities. Most cytological failures in plants exposed to short days occurred late in meiosis and were associated with observable tapetal abnormalities. They do not consider that all pollen sterility is related to tapetal malfunction, since the abnormality may be apparent early, even at the sporogenous mass stage.

SOME PROPOSED CAUSES OF CMS

The Tapetum

This layer often has been implicated as the direct or indirect cause of MMC, microspore, or pollen abortion. According to much of the CMS literature, the tapetum is critical in the abortive process because of its presumed nutritive function during microsporogenesis. Vasil (1967), in a review of anther development, noted that abnormal tapetal behavior caused by factors such as light, heat, drought, and carbohydrate or mineral deficiency is invariably followed by a failure of pollen development. He suggested that there was evidence that tapetal cell degradation products, particularly deoxyribosides, are the main pool for DNA synthesis in normal MMCs and microspores.

In their studies with CMS *Sorghum*, Brooks, Brooks & Chien (1966), Alam & Sandal (1964, 1967), and Narkhede, Phadnis & Thombre (1968) all concluded that a nonfunctional tapetum was the cause of abnormal microspore development and subsequent abortion. In *Triticum*, Chauhan & Singh (1966), Joppa, McNeal & Walsh (1966), and Heyne & Livers (1968) felt that degeneration of the tapetum and its subsequent lack of starch storage was a direct cause of microspore abortion. In *Dactylis*, Filion & Christie (1966) concluded that the tapetum failed to nourish the sporogenous tissue, thus initiating MMC degeneration. Schooler (1967) concluded that, in CMS *Hordeum*, the nutritive tapetum remains intact longer than in N anthers, but that the reason for failure of tapetal breakdown was not known.

Investigators also have regarded the tapetum as a direct cause of microspore degradation in several dicot species. Kinoshita & Nagao (1968) attributed male sterility in *Beta* to a disturbance of nutrient transfer from the degenerating tapetum to the developing microspores. Degradation of the tapetum has been implicated as a direct cause of pollen abortion by Skalinska (1931) in *Aquilegia*, Dubey (1970) in *Tabernaemontana*, and by Novak & Betlach (1970) in *Capsicum*. The effect of some type of abnormal tapetal disintegration or some imbalance during the process of tapetal degradation is reportedly responsible for terminating microspore growth in *Brassica*, *Raphanus*, and *Lycopersicon* (Nishi & Hiraoka, 1958).

Stamen vasculature

Several studies of anther anatomy and morphology have implicated structural changes in the stamen filament, primarily in its vascular bundle, as a plausible explanation of microspore abortion in CMS plants. Anderson (1963) noted that pollen abortion was accompanied by a reduction in anther size and a lengthening of the filament in *Lycopersicon.* Comparisons of normal and CMS *Beta* by Rohrbach (1965) showed that filaments of normal stamens possessed elongated cells with large amounts of cytoplasm, and that cells in filaments of sterile stamens were short, broad, and contained little cytoplasm. He hypothesized that pollen abortion resulted from nutritional imbalances caused by malfunction of the vascular tissue of the stamen.

Joppa, McNeal & Walsh (1966) working with *Triticum* and Alam & Sandal (1967) with sudangrass (*Sorghum*) investigated pollen and anther development in CMS and N plants. They reported that stamens of sterile lines had a poorly differentiated vascular bundle, but that normal stamen vasculature was well differentiated, with both xylem and phloem elements. They concluded that decreased starch accumulation in the tapetum and the lack of starch storage in maturing microspores might be explained by reduced solute transport in stamens of sterile plants.

Laser's (1972) observations using light microscopy, EM and SEM techniques, on the stamen vascular bundle in N and CMS *Sorghum bicolor,* failed to show any structural differences in the vasculature of N and CMS lines during all developmental stages and, thereby, eliminated it as a cause of microspore abortion in sorghum.

Callose Dissolution

A recent paper (Izhar & Frankel, 1971) presents impressive evidence that faulty timing of enzymatic digestion of callose is a primary factor in MMC and microspore abortion in three male sterile lines (2 CMS and one genic MS) of *Petunia hybrida.* After establishing that *in vitro* activity of callase is optimum at about pH 5.0 and inactive above pH 6.3, they measured *in vivo* pH at different stages of microsporogenesis and found that: In the normal, fertile anthers pH is about 7.0 until late tetrad stage, when it drops to around 6.0, followed by callose dissolution. In the RM CMS line pH always remains low, and MMC callose is digested early and abortion occurs early. In the RM genic MS line pH remains high and no callose is dissolved, the microspores therefore aborting while still in tetrads. The PR CMS line showed callase activity delayed until very late tetrad stage, and abortion occurred at the early microspore stage. Their work offers a new insight into the direct cause of abortion.

Viruses

Viruses and their transmission have been investigated extensively in connection with cytoplasmic inheritance and CMS in numerous plant

species. Grafting experiments to show viral transmission as a cause of CMS and subsequent abortion are numerous and have yielded varying results. Comprehensive experimental investigations by Frankel (1956, 1962) and Edwardson & Corbett (1961) have confirmed that graft-induced transmission of viruses conditioning CMS in *Petunia* is possible. Studies in *Humulus* (Cech & Pozdena, 1962) and in *Beta vulgaris* (Curtis, 1967) also demonstrated graft transmissibility of male sterility between CMS and N plants when reciprocally grafted, but only certain plants under proper environmental conditions exhibited positive transmission.

Atanasoff (1971) reviewed numerous studies on viral transmission as the cause of pollen sterility in plants. He also included some original investigations, concluding that viral infections were the sole cause of sterility in species of *Triticum* and *Helianthus*. He maintains that cytoplasmic inheritance and infection genes are a biological impossibility. He further postulates that viral infection symptoms often can be overlooked by the novice and that male sterility is not always characterized by gross morphological defects in the anthers and pollen. He also suspects that faulty techniques and a general lack of understanding of viral infections has resulted in much of the negative results in grafting experiments. His own results, however, do not give convincing proof in support of the viral nature of male sterility.

To our knowledge, no viruses or virus-like inclusions have been shown in CMS anthers. Edwardson (1962) did show electron micrographs of root tip cells of CMS corn with inclusions in "'dense cytoplasmic areas," and similar inclusions were seen in tapetal cells of CMS and maintainer lines. He did not believe that these inclusions could be viruses, because "it seems improbable that only the male-sterile root tips would be virus-infected . . . ."

CONCLUSIONS

The studies reviewed date from 1925 to 1972 and contain extensive anatomical and cytological information all too often incomplete or vague. The terminology for microsporogenesis used is also often sketchy or inaccurate. An attempt therefore has been made to establish some consistency in microsporogenesis terminology via Fig. 1 and the tables. We have given, in convenient tabular form, CMS taxa, investigators, and the morphological and cytological events reported. By referring to a few keys, the reader can gain further insight into specific CMS taxa and can easily compare studies.

The work of Laser (1972) is only part of a more extensive investigation of the anatomy, cytology, and histochemistry of N and CMS *Sorghum bicolor* (Laser, unpub.). To date, only a small part is published (Christensen, Horner & Lersten, 1972), but when completed it probably will be the most complete study to date of these aspects of CMS. Hoefert (1969a, 1969b, 1971) has investigated only normal microsporogenesis so far, but her intention also is to make a detailed descriptive comparison

of N and CMS development. Such comparative electron microscope studies will be needed to help answer questions raised in the Introduction of this review. Concerning the events within microspores at the beginning of abortion, for example, there is complete ignorance of what organelle shows the first sign of disintegration or whether there is a definite sequence or simply a simultaneous collapse. The answer to this question could yield valuable clues to the direct cause of abortion.

Looking at the existing published studies and taking into account numerous examples of questionable technique and interpretation, we conclude that abortion has been shown to occur at almost every point in development, and that probably more than one mechanism is involved.

LITERATURE CITED

ALAM, S. & P. C. SANDAL. 1964. Anther morphology as related to cytoplasmic male-sterility in Sudangrass. Proc. North Dakota Acad. Sci. **18**: 72–73.

———— & ————. 1967. Cyto-histological investigations of pollen abortion in male-sterile Sudangrass. Crop Sci. **7**: 587–589.

ANDERSON, W. R. 1963. Cytoplasmic sterility in hybrids of *Lycopersicon esculentum* and *Solanum penellii*. Tomato Genet. Coop. Rept. **13**: 7–8.

ARTSCHWAGER, E. 1947. Pollen degeneration in male-sterile sugar beets, with special reference to the tapetal plasmodium. J. Agric. Res. **75**: 191–197.

ATANASOFF, D. 1971. The viral nature of cytoplasmic male sterility in plants. Phytopathol. Z. **70**: 306–322.

BROOKS, M. W., J. S. BROOKS & L. CHIEN. 1966. The anther tapetum in cytoplasmic-genetic male sterile *Sorghum*. Amer. J. Bot. **53**: 902–908.

CECH, M. & J. POZDENA. 1962. Untersuchungen uber die infektiose Sterilität des Hopfens. Phytopathol. Z. **44**: 273–281.

CHANG, T. T. 1954. Pollen sterility in maize. M. S. Thesis, Cornell Univ., Ithaca.

CHAUHAN, S. V. S. & S. P. SINGH. 1966. Pollen abortion in male sterile hexaploid wheat (Norin) having *Aegilops ovata* L. cytoplasm. Crop Sci. **6**: 532–535.

———— & ————. 1968. Studies on pollen abortion in *Cucumis melo* L. Agra Univ. J. Res. Sci. **17**: 11–22.

CHOWDHURY, J. B. & T. M. VARGHESE. 1968. Pollen sterility in crop plants—a review. Palynol. Bull. 4: 71–86.

CHRISTENSEN, J. E., H. T. HORNER, JR. & N. R. LERSTEN. 1972. Pollen wall and tapetal orbicular wall development in *Sorghum bicolor* (Gramineae). Amer. J. Bot. **59**: 43–58.

CURTIS, G. J. 1967. Graft-transmission of male sterility in sugar beet (*Beta vulgaris*). Euphytica **16**: 419–424.

DAVIS, G. L. 1966. Systematic embryology of the angiosperms. John Wiley & Sons, Inc., New York. vii + 528 pp.

DE VRIES, A. PH. & T. S. IE. 1970. Electron-microscopy on anther tissue and pollen of male sterile and fertile wheat *Triticum aestivum* L. Euphytica **19**: 103–120.

DIACONU, P. 1965. (Cytological investigations on corn cytoplasmic male sterility.) Amelior., Genet. Fiziol. Technol. Agr. **33**: 227–228. (Russian with English summary).

DUBEY, D. K. & S. P. SINGH. 1965. Mechanism of pollen abortion in three male sterile lines of flax (*Linum usitatissimum L.*). Crop Sci. **5**: 121–124.

DUBEY, R. S. 1970. Pollen abortion in crape-jasmine. Indian J. Hort. **27**: 54–56.

———— & S. P. SINGH. 1969. Pollen abortion in chemically induced male-sterile coriander. J. Indian Bot. Soc. **48**: 118–124.

EDWARDSON, J. R. 1962. Cytoplasmic differences in T-type cytoplasmic male-sterile corn and its maintainer. Amer. J. Bot. **49**: 184–187.

———. 1967. Cytoplasmic male sterility and fertility restoration in *Crotalaria mucronata*. J. Heredity **58**: 266–268.

———. 1970. Cytoplasmic male-sterility. Bot. Rev. (Lancaster) **36**: 341–420.

——— & M. K. CORBETT. 1961. Asexual transmission of cytoplasmic male sterility. Proc. Natl. Acad. Sci. U. S. A. **47**: 390–396.

——— & H. E. WARMKE. 1967. Fertility restoration in cytoplasmic male-sterile petunia. J. Heredity **58**: 195–196.

ERICHSEN, A. W. & J. G. ROSS. 1963. Inheritance of colchicine induced male sterility in *Sorghum*. Crop Sci. **3**: 335–338.

FEDOROVA, T. N. & E. D. NETTEVICH. 1969. (Study of microsporogenesis in forms of common wheat with male sterility specified by the cytoplasm of some species.) Cytologiya **11**: 1121–1128. (Russian with English summary).

FILION, W. G. & B. R. CHRISTIE. 1966. The mechanism of male sterility in a clone of orchard grass (*Dactylis glomerata* L.). Crop Sci. **6**: 345–347.

FRANKEL, R. 1956. Graft induced transmission to progeny of cytoplasmic male sterility in *Petunia*. Science **124**: 684–685.

———. 1962. Further evidence of graft induced transmission to progeny of cytoplasmic male sterility in *Petunia*. Genetics **47**: 641–646.

FUKASAWA, H. 1953. Studies on restoration and substitution of nucleus in *Aegilotricum*. I. Appearance of male-sterile durum in substitution crosses. Cytologia **18**: 167–175.

———. 1956. Studies on restoration and substitution of nucleus (genome) in *Aegilotricum*. III. Cytohistological investigation of pollen degeneration in anthers of male-sterile plants. Cytologia **21**: 97–106.

GABELMAN, W. H. 1949. Reproduction and distribution of the cytoplasmic factor for male sterility in maize. Proc. Natl. Acad. Sci. U. S. A. **35**: 634–640.

GRUN, P. & M. AUBERTIN. 1966. Cytological expressions of a cytoplasmic male sterility in *Solanum*. Amer. J. Bot. **53**: 295–301.

HERICH, R. 1965. Nucleoli and cytoplasmic male sterility. Z. Vererbungsl. **96**: 22–27.

HESLOP-HARRISON, J. 1957. The experimental modification of sex expression in flowering plants. Biol. Rev. Cambridge Phil. Soc. **32**: 38–90.

——— & Y. HESLOP-HARRISON. 1958. Long-day and auxin induced male sterility in *Silene pendula* L. Port. Acta Biol. **5**: 79–94.

HEYNE, E. G. & R. W. LIVERS. 1968. Use of male sterility in the breeding of self pollinating crops. Proc. XII Int. Cong. Genet. 230–231.

HOEFFERT, L. L. 1969a. Ultrastructure of *Beta* pollen. I. Cytoplasmic constituents. Amer. J. Bot. **56**: 363–368.

———. 1969b. Fine structure of sperm cells in pollen grains of *Beta*. Protoplasma **68**: 237–240.

———. 1971. Ultrastructure of tapetal cell ontogeny in *Beta*. Protoplasma **73**: 387–406.

HORNER, H. T., JR. & N. R. LERSTEN. 1971. Microsporogenesis in *Citrus limon* (Rutaceae). Amer. J. Bot. **58**: 72–79.

HOSOKAWA, S., T. TAKEDA, Y. OTANI & M. IKEHATA. 1954. Cyto-histological studies on male sterility of sugar beets with special reference to pollen degeneration and tapetal plasmodium. Jap. J. Breed. **4**: 196–202.

IZHAR, S. & R. FRANKEL. 1971. Mechanism of male sterility in *Petunia*: The relationship between pH, callase activity in the anthers, and the breakdown of the microsporogenesis. Theor. Appl. Genet. **44**: 104–108.

JONES, D. F., H. T. STINSON, JR. & U. KHOO. 1957. Pollen restoring genes. Connecticut Agric. Exp. Sta. Bull. Immed. Inform. 610.

JONES, H. A. & L. K. MANN. 1963. Onions and their allies. Interscience Publishers, New York. 286 pp.

JOPPA, H. A., F. H. MCNEAL & J. R. WALSH. 1966. Pollen and anther development in cytoplasmic male sterile wheat *Triticum aestivum* L. Crop Sci. **6**: 296–297.

KAUL, C. L. & S. P. SINGH. 1967. Induction of male sterility in *Allium cepa* L. Curr. Sci. **36**: 676–677.

KHOO, U. & H. T. STINSON, JR. 1957. Free amino acid differences between cytoplasmic male sterile and normal fertile anthers. Proc. Natl. Acad. Sci. U. S. A. **43**: 603–607.

KIDD, H. J. 1961. The inheritance of restoration of fertility in cytoplasmic male-sterile sorghum—a preliminary report. Sorghum Newslett. **4**: 47–49.

KINOSHIRA, T. & S. NAGAO. 1968. Use of male sterility in triploid sugar beets. Proc. XII. Int. Cong. Genet. 232–233.

——— & ———. 1966. Inheritance of pollen sterility induced by the irradiation. Bull. Sugar Beet Res. Suppl. **7**: 40–42.

LAMM, R. 1941. Varying cytological behavior in reciprocal *Solanum* crosses. Hereditas **27**: 202–208.

LASER, K. D. 1972. A light and electron microscope study of the stamen vascular bundle in cytoplasmic male sterile and normal *Sorghum bicolor*. Amer. J. Bot. **59**: 653 (abstract).

———. (unpublished) A light and electron microscope study of microsporogenesis in cytoplasmic male sterile *Sorghum bicolor*. Ph.D. Thesis, Iowa State Univ., Ames.

MAUNDER, A. B. & R. C. PICKETT. 1959. The genetic inheritance of cytoplasmic-genetic male sterility in grain *Sorghum*. Agron. J. **51**: 47–49.

MONOSMITH, H. R. 1928. Male sterility in *Allium cepa* L. Ph.D. Thesis. Univ. Calif. (Not seen; cited by Jones & Mann, 1963).

MOSS, G. I. 1967. A cytochemical study of DNA, RNA, and protein in the developing maize anther. I. Methods. Ann. Bot. **31**: 545–553.

——— & J. HESLOP-HARRISON. 1967. A cytochemical study of DNA, RNA, and protein in the developing maize anther. II. Observations. Ann. Bot. **31**: 555–574.

——— & ———. 1968. Photoperiod and pollen sterility in maize. Ann. Bot. **32**: 833–846.

NAGAO, S. & T. KINOSHITA. 1962. Causal agents and character expression of male sterility in beets. J. Fac. Agric. Hokkaido Univ. **52**: 51–69.

NARKHEDE, M. N., B. A. PHADNIS & M. V. THOMBRE. 1968. Cytological studies in some male sterile Jowars (*Sorghum vulgare* Pers.) and their maintainers and restorers. Cytologia **33**: 168–173.

NISHI, S. & T. HIRAOKA. 1958. (Histological studies on the degenerative process of male sterility in some vegetable crops.) Bull. Natl. Inst. Agric. Sci. Japan, Ser. E, No. 6. (Japanese with English summary).

NITSCHE, W. 1971. Cytoplasmatische mannliche Sterilität bei Weidelgras (*Lolium* sp.). Z. Pflanzenzucht. **65**: 206–220.

NOVAK, F. 1971. Cytoplasmic male sterility in sweet pepper (*Capsicum annuum* L.) II. Tapetal development in male sterile anther. Z. Pflanzenzucht. **65**: 221–232.

——— & J. BETLACH. 1970. Development and karyology of the tapetal layer of anther in sweet pepper (*Capsicum annuum L.*) Biol. Plantarum **12**: 275–280.

———, ——— & J. DUBOVSKY. 1971. Cytoplasmic male sterility in sweet pepper. I. Phenotype and inheritance of male sterile character. Z. Pflanzenzucht. **65**: 129–140.

NÜRNBERG-KRÜGER, U. 1956. Die Beeinflussung des Geschlechts bei zwittrigen *Fragaria*-Arten durch Bastardierung. Ber. Deutsch Bot. Ges. **68**: 16.

———. 1958. Genetische Untersuchungen und Diploiden *Fragaria*-Arten, I. Die Kreuzung zwischen *F. vesca* und *F. nilgerrensis*, ihre Auswirkung auf

die Morphologie der Pflanzen und die Ausbildung des Geschlechts. Z. Vererbungsl. **89**: 747–773.

OBERREUTER, M. 1925. Untersuchung der Pollensterilität der reziprok verschiedenen Epilobiumbastarden. Ber. Deutsch Bot. Ges. **43**: 47–51.

OGURA, H. 1968. Studies on the new male-sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. Mem. Fac. Agric. Kagoshima Univ. **6**: 39–78.

OREL, L. I. 1967. (A cytological study of maize pollen with cytoplasmic male sterility.) Genetika **12**: 3–11. (Russian with English summary.)

PAKENDORF, K. W. 1970. Male sterility in *Lupinus mutabilis* Sweet. Z. Pflanzenzucht. **63**: 227–236.

PALMER, R. 1971. Cytological studies of ameiotic and normal maize with reference to premeiotic pairing. Chromosoma (Berl.) **35**: 233–246.

PETERSON, P. A. 1958. Cytoplasmically inherited male sterility in *Capsicum*. Amer. Natur. **90**: 111–119.

RAJ, A. Y. 1968. Histological studies in male sterile and male fertile *Sorghum*. Indian J. Genet. Pl. Breed. **28**: 335–341.

RHOADES, M. M. 1933. The cytoplasmic inheritance of male sterility in *Zea mays*. J. Genet. **27**: 71–95.

ROGERS, J. S. & J. R. EDWARDSON. 1952. The utilization of cytoplasmic male sterile inbreds in the production of corn hybrids. Agron. J. **44**: 8–13.

ROHRBACH, U. 1965. Beitrage zum Problem der Pollensterilität bei *Beta vulgaris* L. I. Untersuchungen uber die Ontogenese des Phanotyps. Z. Pflanzenzucht. **53**: 105–124.

SAVCHENKO, N. I. 1967. (Microsporogenesis and pollen grain development in cytoplasmic male sterile lines of winter wheat). Citol. Genet. **1**: 28–37. (Russian with English summary).

SAVCHENKO, N. I. & A. S. LASTOVYCH. 1965. (Morphological and cytological peculiarities of wheat forms with cytoplasmic male sterility). Ukrajins' K. Bot. Zurn. **22**: 35–42. (Russian with English summary).

SCHOOLER, A. B. 1967. A form of male sterility in barley hybrids. J. Heredity **58**: 206–211.

SINGH, S. P. & H. H. HADLEY. 1961. Pollen abortion in cytoplasmic male sterile sorghum. Crop Sci. **1**: 430–432.

———— & Y. P. SHARMA. 1963. Preliminary observations on the breeding of *Pennisetum* at B. R. College, Bichpuri, Agra, India. Sorghum Newslett. **6**: 26–28.

SKALINSKA, M. 1928. Sur les causes d'une disjunction non typique des hybrides du genre *Aquilegia*. Acta Soc. Bot. Poloniae **5**: 141–173.

————. 1931. A new case of unlike reciprocal hybrids in *Aquilegia*. Rep. Fifth Int. Bot. Congr., p. 250.

SPASOJEVIC, V. 1966. Contribution to the study of cytoplasmic male sterility in maize. J. Sci. Agric. Res. **19**: 30–42.

STEPHENS, J. C. 1937. Male sterility in sorghum: Its possible utilization in production of hybrid seed. J. Amer. Soc. Agron. **29**: 690–696.

TATEBE, T. 1957. Cytological studies on pollen degeneration in male-sterile onions. J. Hort. Assoc. Japan **21**: 73–75. (Japanese with English summary).

THOMPSON, D. J. 1960. Studies on the inheritance of male-sterility and other characters in the carrot, *Daucus carota* L. var. *sativa*. Proc. Am. Soc. Hort. Sci. **78**: 332–338.

TSIKOVA, E. 1969. Cytological investigations on the cytoplasmic male sterility in *Nicotiana*: IV. Changes in the tapetum. Genet. Pl. Breed. **2**: 461–467.

TURBIN, N. V., A. N. PALILOVA, A. V. SMOL'SKAYA & L. S. SERPOKRYLOVA. 1969. The influence of sterile cytoplasm on the course of microsporogenesis in maize lines. Dokl. Akad. Nauk SSSR. **188**: 108–111.

VASIL, I. K. 1967. Physiology and cytology of anther development. Biol. Rev. Cambridge Phil. Soc. **42**: 327–373.

VIRNICH, H. 1967. Untersuchungen über das verhalten der männlichen sterilität und anderer Eigenschaften bei polyploiden Zweibeln (*Allium cepa* L.) als Grundlage für eine Nutzung in der Hybridüchtung. Z. Pflanzenzucht. **58**: 205–244.

WILSON, J. A. 1968. Problems in hybrid wheat breeding. Euphytica **17**: 13–33.

YAMAGUCHI, T. & C. KANNO. 1963. (Studies on the differences of character manifestation in reciprocal crosses of Rape (*Brassica napus* L.) VI.) 1st Agron. Div. Tokai-Kinki Natl. Agric. Exp. Sta. Bull. No. 9: 162–182. (Japanese with English summary).

ZENKTELER, M. 1962. Microsporogenesis and tapetal development in normal and male sterile carrots (*Daucus carota*). Amer. J. Bot. **49**: 341–348.