STUDIES WITH CYTOPLASMIC MALE-STERILE RYE

A Thesis

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by

Graham John Scoles

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of

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FOREWORD

This thesis is written in the paper style, specified in the 1976 Plant Science Thesis Preparation Guide. It contains four manuscripts. The first, entitled "The transfer of a cytoplasmic male-sterility system to spring rye", was published in the Canadian Journal of Plant Science, Volume 59, pages 163-169. The second, entitled "The effect of temperature on pollen fertility and anther dehiscence of cytoplasmic male-sterile rye", is to be published in the July 1979 issue of the Canadian Journal of Plant Science. The third, entitled "The genetics of fertility restoration in cytoplasmic male-sterile rye" is currently under review by the Canadian Journal of Genetics and Cytology. The fourth paper, entitled "Pollen development in male-fertile and cytoplasmic male-sterile rye" will be submitted to the Canadian Journal of Botany.

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Scoles, Graham John. Ph.D., The University of Manitoba, October 1979. Studies with cytoplasmic male-sterile rye. Major Professor: Dr. L.E. Evans.

An attempt was made to transfer cytoplasmic male-sterility from winter rye (Secale cereale L.) to six inbred lines of spring habit and to an open-pollinated spring cultivar. All except one of the inbred lines and the open-pollinated cultivar exhibited segregation for fertility restoration. The other inbred line restored full fertility to all plants. Through further inbreeding sub-lines either homozygous for maintenance of sterility or for restoration of fertility were obtained from some inbred lines. During this process environmental effects on the expression of fertility restoration were detected. Attempts to inbreed and select from the open-pollinated cultivar were unsuccessful due to high self-sterility and the high mortality of any selfed progeny.

Pollen fertility and anther dehiscence of two cytoplasmic male-sterile lines, their maintainers, their restorers and the F_1 between each sterile and restorer were investigated at three temperature regimes (15/10, 20/15 and 25/20°C). The anther dehiscence of five additional sterile/restorer hybrids was investigated at the same temperatures. Anthers of male-sterile plants did not contain pollen grains and were non-dehiscent at all temperatures. Pollen fertility

of maintainer, restorer and sterile/restorer hybrids varied with temperature. All anthers of maintainer and restorer lines were fully dehiscent, but partially dehiscent and non-dehiscent anthers occurred in the sterile/restorer hybrids. Anthers of florets in the upper and lower portions of spikes of the sterile/restorer hybrids were often partially dehiscent or non-dehiscent. Variation among tillers of a plant with respect to this character was low, but variation among plants of a sterile/restorer hybrid was high suggesting genetic segregation. The classification of an anther as either dehiscent, partially dehiscent or non-dehiscent was directly related to pollen fertility. Better restoration of fertility was obtained at temperatures of 20/15 or 25/20°C than at 15/10°C.

Three inbred lines known to be capable of restoring fertility were crossed with the sterile lines. The proportions of male-fertile, partially male-fertile and male-sterile plants in F_2 and backcross progenies indicated that three dominant restorer genes were present in each line. These were designated Rf_1, Rf_2 and Rf_3. Their relative expressivity was $Rf_1 > Rf_2 > Rf_3$. Partial fertility occurred when certain genotypes carried two of the three alleles as dominant, but this was dependent upon genotype and environment.

Pollen development in a male-fertile and a cytoplasmic male-sterile line was investigated through the use of anther sections. In the male-fertile line a high degree of organization was evident within the locule, and polarity within the microspore was also evident. In the male-sterile line development appeared to proceed normally until the tetrad stage. Just after tetrad break-up the tapetum became vacuolated and invaded the locule. Two days later the organization within the

locule had broken down completely. Microspores and tapetum had become an unorganized mass within the locule. By 10 days after tetrads the middle layer had also broken down. At dehiscence the contents of the locule remained as a compressed layer over the endothecium of the anther.

INTRODUCTION

In the last decade there have been a number of reports documenting the occurrence in rye of cytoplasmic male-sterility (see Literature Review). One form of sterility, developed by Geiger (1970) was a result of the incorporation of the cytoplasm of an Argentinian rye cultivar "Pampa" into German material. Lines carrying this sterility were distributed to plant breeders throughout the world (Geiger, personal communication). Despite the wide distribution of this material there have been no reports as to the genetics of restoration, and none referring to the effect of environment on sterility or fertility restoration. One report (Garlicka and Madej, 1975) included lines carrying this sterility as part of a histological investigation of pollen abortion.

A report by the Canada Grains Council (1970) recognized rye as the "preferred grain crop for extensive areas (of Canada), especially for rotation purposes with other cereals". This realization prompted renewed interest in rye, and was responsible for the initiation of research directed at attempting to identify feeding problems associated with rye. If this is successful, an increase in the demand for rye could be expected and an enlarged rye breeding program would be warranted. Such a program might successfully utilize a source of cytoplasmic male-sterility. In view of this possibility the objectives

of this study were four: i) to investigate the feasibility of incorporating cytoplasmic male-sterility into Canadian material, ii) to investigate the effects of temperature on sterility and fertility restoration, iii) to investigate the genetics of restoration, and iv) to investigate pollen abortion in sterile lines.

LITERATURE REVIEW

Cytoplasmic Male-Sterility in Rye

The Development of Cytoplasmic Male-Sterility and its Restoration

Putt (1954) undertook a study of the cytogenetics of sterility in rye (Secale cereale L.) using a number of families obtained from the crossing of an inbred line derived from a German cultivar and an inbred line derived from the cultivar Minnesota 104. In one family differences in the male-fertility of the progenies of a reciprocal cross indicated cytoplasmic control of pollen abortion. No further work was carried out with this material, the first reported to exhibit cytoplasmic male-sterility in rye.

Chekhovskaja (1965) and Zdril'ko (1966) published reports of cytoplasmic male-sterility in rye. Dmitrieva and Zdril'ko (1967) reported finding cytoplasmic male-sterile plants in a line of spring rye, VIR 9627. Zdril'ko (1969) transferred this sterility to six winter ryes, none of which were complete maintainers for the sterility. Zdril'ko (1972) reported further on transferring the sterility to 67 winter ryes of which seven were non-restorers. Lines were isolated from partial restorers which gave 100% restoration.

Kobyljanskij (1968 and 1969) studied male-sterility in populations of winter rye. Of 1300 lines examined, 150 exhibited some male-

sterility. These were mostly older cultivars or weedy ryes. In certain cases the male-sterility seemed to be cytoplasmically inherited. When sterile plants were pollinated by fertile lines, progenies consisted of 0-70% fertile plants. Among the fertile lines, restorers of the fertility were found to be more frequent than maintainers. Analysis of the progeny from crosses of sterile by restorer and from self-pollination of restorers indicated that restoration of fertility was controlled by a single gene. Kobyljanskij (1971) reported on a second type of cytoplasmic male-sterility in which restoration was brought about by more than one gene. Of 145 cultivars tested in crosses with a male-sterile 66 resulted in 1-20% steriles, 46 gave 21-40% steriles, 24 gave 41-60% sterile progeny, 8 gave 61-80% steriles and one gave over 81% sterile progeny. By selecting for sterile progeny over a number of generations male-sterile analogues of 70 cultivars were obtained.

Kobyljanskij and Katerova (1973a) examined the F1 and F2 progenies of 30 cytoplasmic male-sterile lines crossed to restorer lines. In most cases restoration was controlled by a single dominant gene. Katerova (1975) crossed 40 cytoplasmic male-sterile lines to a restorer line. In 26, segregation in the F2 suggested monogenic control. In five, polygenic control or multiple alleles of one gene were implicated in restoration. In the remaining nine, segregation in the F2 suggested monogenic inheritance but the test-cross did not result in a 1:1 ratio, there being fewer steriles than expected.

Zdril'ko and Adamchuk (1975) analyzed 97 hybrid progenies obtained by crossing male-fertile and male-sterile analogues of a number of winter rye cultivars. They concluded that restoration was controlled

by a polygenic complex of complementary genes. Restoration required all genes to be dominant, the presence of one recessive gene causing less than full fertility.

Geiger and Schnell (1970) discovered another source of cytoplasmic male-sterility in rye. An inbred line of the German cultivar Petkus had been crossed to the Argentinian cultivar, Pampa. Progeny from this cross were selfed and the resulting S1 lines were grown in the field. In five progenies various percentages of male-sterile plants were observed. These sterile plants were either sibbed or allowed to out-pollinate and the progeny were used in a top-cross program to four inbreds. Fertile and sterile plants in each top-cross progeny were backcrossed to their respective inbreds.

From this material Geiger and Schnell (l.c.) were able to conclude that the male-sterility was cytoplasmically inherited. The variety Pampa seemed to carry restorer gene(s) for the sterility as did one of the inbreds. The other three inbreds used gave rise to predominantly male-sterile progeny suggesting that they were non-restorer types. Observation of the segregation that occurred in the five initial progenies that exhibited male-sterility suggested that sterility was controlled by more than one gene. Environmental effects on the stability of the sterility over years or between glasshouse and field appeared negligible. Geiger (1972) crossed the developed cytoplasmic male-sterile line to 13 highly inbred lines. Two lines proved to be fully effective as restorers, eight lines were classified as non-restorers and the remaining three lines segregated.

Geiger (1971) discovered another source of cytoplasmic male-sterility in an open-pollinated population of a primitive Iranian

rye. By crossing plants of this rye as female to a non-restorer line of the Pampa source of sterility this second source of sterility was isolated.

Geiger and Morgenstern (1975) carried out further studies with these two sources of cytoplasmic male-sterility. Restorer test-crosses of ten open-pollinated cultivars to a cytoplasmic male-sterile line carrying the Pampa source of male-sterility resulted in 28% male-fertile, 27% partially male-fertile and 45% male-sterile progeny. The restoring ability varied among the ten cultivars from 20-50% fertile progeny. In the next year progeny resulting from crosses of the same ten cultivars to another cytoplasmic male-sterile line, also carrying the Pampa source of male-sterility, were investigated. No line resulted in more than 20% fertile progeny, however, the number of male-sterile plants was similar to that in the previous year whereas the number of partially male-fertile plants had increased. This was attributed to environmental differences between the two years and to differences between the two tester lines used.

The same two tester lines were also used in restorer test-crosses with a number of inbred lines derived from German cultivars. In the first year 87 inbred lines were crossed to one of the tester lines, in the next year the same 87 inbreds and another 110 inbreds were crossed to the other tester line. In the first year 46 of the inbreds were classified as non-restorers, three as restorers and the rest as partial restorers. In the second year, 41 of the 197 lines were classified as non-restorers and only one as a full restorer. Eighteen lines were classified as non-restorers in both years, but no line was classified as a restorer in both years.

Geiger and Morgenstern (l.c.) reported that 15 sources of cytoplasmic male-sterility in rye had been identified by them. Four forms traced back to the Pampa cytoplasm, ten to the Iranian rye cytoplasm and one to the cytoplasm of some European material. A test of six genotypes with three sources of cytoplasmic male-sterility (one Pampa and two Iranian) was carried out. Three genotypes gave identical reactions with all three cytoplasms. The other three genotypes gave similar results with the two Iranian sources of cytoplasmic male-sterility, but gave a different result with the Pampa cytoplasm suggesting a plasmotype/genotype interaction.

Klyuchko and Belousov (1972) reported the discovery of cytoplasmic male-sterile lines in the cultivars Mestnaya, Kharkov 55, Odessa 1 and Viglasska. Restoration seemed to be controlled by a single gene. Gulyaeva (1972) analysed male-sterile plants that occurred in a population of Petkus rye. In some of these the sterility was also classified as cytoplasmic. Analysis of a number of rye lines showed them to contain 12.8-19.1% sterility maintainers.

Lapinski (1972) has reported the development of cytoplasmic male-sterility by crossing cultivated rye with the perennial species Secale montanum Guss. Reciprocal crosses were made and the two backcross progenies were developed. Substitution of the S. montanum nucleus into S. cereale cytoplasm resulted in cytoplasmic male-sterility in six of seven lines. The remaining plants and plants of the reciprocal backcross were male-fertile. Further testing demonstrated the cytoplasmic nature of the sterility and genetic studies suggested that restoration was due to one dominant gene carried by

certain clones of S. montanum. Lapinski (l.c.) also reported that substitution of the S. kuprijanovii Grossh. nucleus into S. cereale cytoplasm resulted in sterility which could be restored by one dominant gene from S. kuprijanovii.

Madej (1975) analysed three sources of cytoplasmic male-sterility and a number of restorer lines. The three sources were designated L1P (from Geiger), Smol. 128/3 derived by Madej from the cultivar Smolickie and Wcms (from Kobyljanskij). From a number of inbred lines several complete fertility restorers were found for Smol. 128/3 and Wcms but only two were found for L1P. No complete sterility maintainers were found for Wcms. Line LN1, the maintainer line for L1P restored fertility in both Smol. 128/3 and Wcms. Two lines which maintained the sterility of Smol. 128/3 restored fertility in L1P and Wcms.

The Development of Pollen in Cytoplasmic Male-Sterile Rye

Belousov and Klyuchko (1970) carried out comparative cytological studies of cytoplasmic male-sterile and male-fertile forms. Meiosis in the sterile forms was found to be normal. Degeneration of the male gametophyte occurred during the early development of the microspore. In a further study (Klyuchko and Belousov, 1970) the start of pollen degeneration in one line was observed between formation of the germination pore and development of the vacuole. In other lines it occurred at a later stage. Orel (1972), also found that pollen development in cytoplasmic male-sterile rye was normal until the early development of the microspores. After vacuolization, lysis of the microspore occurred. Gulyaeva (1972) found that degeneration occurred after formation of the germination pore.

Kobyljanskij and Katerova (1973b) compared pollen development in cytoplasmic male-sterile forms with that of a genetic male-sterile. In the cytoplasmic male-steriles meiosis was normal, microspore degeneration beginning after meiosis. In the genetic male-sterile degeneration began in the early stages of formation of the pollen mother cells. Zdril'ko and Adamchuk (1975) were able to classify cytoplasmic male-steriles into two groups. In one group microspores degenerated at the uninucleate stage, whereas in the other group degeneration did not begin until after this stage. Such differences between sterile lines were also reported by Klyuchko and Belousov (1970).

Garlicka and Madej (1975) studied meiosis and pollen development in one fertile and two cytoplasmic male-sterile lines of rye using anther sections obtained throughout pollen development. Differences between fertile and steriles were not found until after the break-up of tetrads. In the fertile line, central vacuolization of the microspore and movement of the nucleus towards the cell-wall occurred. In both sterile lines microspore degeneration began prior to vacuolization, indicated by separation of the cytoplasm from the cell-wall, its gradual contraction led to complete deterioration and the production of an irregularly shaped, empty pollen grain. Observation of the tapetal layer revealed that in fertile anthers this layer underwent degeneration during early pollen development. In both sterile lines, however, degeneration of the tapetum was delayed such that when only vestiges of the tapetum remained in the fertile line; in the sterile lines the tapetum still retained its cellular nature. The relationship between microspores and tapetum also seemed to differ between

fertile and steriles. In the fertile the microspores retained close contact with the tapetum until anthesis. In both sterile forms microspores lost contact with the tapetum soon after the end of meiosis. In one sterile line this disturbance was observed as early as tetrads.

Cytoplasmic Male-Sterility in Other Members of the Gramineae

An extensive review of cytoplasmic male-sterility in plant species was carried out by Edwardson (1970). He documented the occurrence of cytoplasmic male-sterility in 153 species of 51 genera and 22 families. This present review of cytoplasmic male-sterility in the Gramineae is limited to the three areas dealt with in this thesis; the genetics of restoration, the development of pollen in male-steriles and the stability of restoration.

The Genetics of Restoration

Wheat. The first cytoplasmic male-sterile forms of wheat (Triticum aestivum L. em. Thell.) were developed using backcross techniques to place the wheat nucleus in the cytoplasm of various Aegilops species (Kihara, 1951; Fukasawa, 1953). Recent work on cytoplasmic male-sterility in wheat has utilized forms carrying the wheat nucleus in the cytoplasm of Triticum timopheevi Zhuk., first developed by Wilson and Ross (1962).

Wilson (1962, cited by Robertson and Curtis, 1967) suggested that restorer genes for the sterility induced by timopheevi cytoplasm might be present in T. timopheevi itself. In that same year Wilson, and also Schmidt et al. (1962) reported the recovery of fertility restoring genes from material derived from T. timopheevi.

The genetics of restoration of fertility has been studied by analysis of F2 and test-cross progenies, or by the use of monosomic analysis. The results of studies with a number of restorers are presented in Table 1. The number of restorer genes reported to be carried by a restorer varies from one to more than three. To date seven chromosomes have been found to carry a dominant gene for restoration of fertility to sterile lines carrying timopheevi cytoplasm.

Through monosomic analysis a number of chromosomes have been found to have minor effects on restoration (Table 1). The progeny of plants carrying these chromosomes of the restorer in a monosomic condition contain a greater than expected number of steriles. Robertson and Curtis (1967) assumed that this increase in sterility was due to the absence of the non-restorer chromosome from the monosomic plant. It was suggested that these particular non-restorer chromosomes carry modifying genes which collectively have a complementary epistatic effect on the major restorer genes and whose presence is critical to full restoration. Alternatively, Bahl and Maan (1973) have suggested that these chromosomes of the restorer line carry genes which reduce the penetrance of the major restorer genes.

From Table 1 it can be seen that there has been disagreement as to the number of fertility restorer genes carried by some restorers. There is now evidence that certain restorer genes are not always fully expressed except under particular environments, while others are more stable. Talaat et al. (1973) found that certain restorer genes were best detected in the "fertile" environment of Obregon, Mexico, e.g. the gene on chromosome 5A of R1-Lee and R4. Similarly, the modifying genes were best detected in the "sterility favouring" environment of

ADDENDUM TO TABLE I

Key to References Used

Reference Code	Reference
1	Anderson, 1964
2	Bahl and Maan, 1973
3	Bajwa and Lucken, 1968
4	Groujon and Ingold, 1967
5	Hughes and Bodden, 1977
6	Livers, 1964
7	Mihaljev, 1973
8	Miller and Schmidt, 1970
9	Miller, Schmidt, and Johnson, 1973
10	Robertson and Curtis, 1967
11	Tahir and Tsunewaki, 1969
12	Talaat, 1969
13	Talaat, Maan, and Lucken, 1968
14	Talaat, Maan, and Lucken, 1973
15	Yen, Evans, and Larter, 1969
16	Zeven, 1972

TABLE 1. Number and chromosomal location of genes which restore or modify the fertility of wheat carrying *timopheevi* cytoplasm

Restorer line	Location restorer genes†	Location of modifier genes	Reference code
R1-Lee	Three genes		3
	1A 5A	1B	13
	1A 7D > 5A	Not 1B	12
	1A > 7D + 1 other	3A 4A 5B 2D 3D 4D	2
	1A 7D 5A	1B 4B 5B	14
R2-Sonora	1A > 6B = 7D	1B	2
R3	Two genes		1
	Two genes		6
	1A + 1 other	2A 6A 1B 6B 3D	10
	Two genes		3
	1A 7D		13
	1A 7D	1B	12
	1A > 7D	4A 5A 5B	2
	1A 7D	1B	14
R4	Two genes		3
	1A 7D 5A	1B	13
	1A > 7D	6A	15
	1A 7D > 5A	1B,	12
	1A 7D	4A 6A 7A 5B	2
	1A 7D > 5A	7A 1B 5B 6B	14
R5	7D > 1A = 7B	3A 5A 6A 3B 5B 3D	2
Primepi	One gene		4
	Two genes		8
	1B > 5D		2
	Two genes	3A 5B 2D 4D	9
Canthatch	6B > 6D	2A 3D	15
Karn	1A 6B	6A	15
T. spelta var. *duhamelianum*	1B	7D	11
Minister	1B	4B 7D	16
YK-64-28	More than three genes		7
Five advanced lines	One gene		5

†, > and = indicate the order of expressivity of restorer genes

N. Dakota, e.g. genes on 4A and 5B of R1-Lee. Talaat et al. (l.c.) also found that the expressivity of restorer genes and modifiers was not equal or constant over environments. Bahl and Maan (1973) ranked the fertility restoration genes present in a restorer in order of expressivity. Expressivity was found to vary depending on genotype and their results are indicated in Table 1.

Hughes and Bodden (1977) crossed five advanced lines of wheat onto male-sterile cultivars. None of nine F2 families from the five crosses deviated significantly from a ratio of three fertile to one sterile, suggesting control by a single dominant gene. Under field conditions this one gene gave good restoration, spike fertility of the five crosses ranged between 90 and 98%.

Tsunewaki (1974) summarized the chromosomal location of fertility restoring genes against various male-sterile cytoplasms. He included seven genes which restore fertility to timopheevi cytoplasm, and five genes which restore fertility to plants carrying the cytoplasm of various Aegilops species. Of these twelve genes, Tsunewaki noticed that seven occur on chromosomes that carry a nucleolus organizer (chromosomes 1A, 1B, 6B and 5D restore to timopheevi cytoplasm while 1B, 1C and 6B restore to Aegilops cytoplasm). He suggested that this was more than would be expected by chance and proposed that the nucleolus organizing region, or the nucleolus itself, plays an important role in fertility restoration. It was noted that in the cultivar Salmon, the loss of a satellite was associated with the loss of a restorer gene.

Tsunewaki (1974) also noted that all except two of the twelve restorer genes were carried by chromosomes of homoeologous groups

1, 6 and 7. Tahir (1971) reported that gene Rf3 on chromosome 1B had a fertility restoration spectrum against a number of cultivars similar to gene Rfc, located on chromosome 1C. Tsunewaki (1974) suggested that these results indicated the homoeologous nature of the fertility restoring genes on chromosomes of the same homoeologous group.

Corn. Cytoplasmic male-sterility in corn (Zea mays L.) has been reviewed by Duvick (1965). In this review he documented a total of 84 separate discoveries of cytoplasmic male-sterility in corn. Most sources of cytoplasmic male-sterility were initially identified in segregating populations, with the exception of two sources found in inbred lines which exhibited differential fertility in reciprocal crosses. Unlike wheat the cytoplasmic male-sterility was not manufactured deliberately (Duvick, 1965). One source of male-sterility, known as Texas or T-cytoplasm, and discovered by Rogers and Edwardson (1952) was widely utilized in the hybrid corn program of the U.S.A. Ullstrup (1972) reports that 85% of the hybrid corn seed produced in the U.S.A. in 1970 carried this cytoplasm. However, in the years 1969, 1970 and 1971 an epiphytotic outbreak of southern leaf blight (Helminthosporium maydis Nisikada and Miyake) race T occurred. This race was found to be virulent only on plants carrying the Texas cytoplasm and led to attempts to diversify the sources of cytoplasmic male-sterility used in the hybrid corn program.

The genetics of fertility restoration were first investigated with the Texas (T) cytoplasmic male-sterility developed by Rogers and Edwardson (1952). Edwardson (1955) concluded that a single dominant

gene was responsible for fertility restoration, but his results also suggested the possibility of a second major gene and the presence of other "modifying" genes. Duvick (1956) proposed that the modifier genes were essential to full restoration in all except the most favourable environments. Duvick (1959) confirmed the existence of two dominant genes for restoration, Rf1 and Rf2, either of which if present as a homozygous recessive could cause sterility. Rf1 and Rf2 have been located on the long arm of chromosome 3 (near the centromere) and on the short arm of chromosome 9 respectively (Duvick, 1965). The number and location of modifier genes is not known, although Beckett (1966), using lines carrying marked chromosomes, detected genes affecting restoration on chromosomes 2, 3, 7 and 9 of one inbred and on chromosomes 2, 3, 4, 7 and 10 of another. A number of alleles at the Rf1 locus have been detected in certain inbred lines (Duvick, 1965).

The genetics of restoration to USDA cytoplasmic male-sterility (developed by Jones et al., 1957b) has not been as extensively investigated as those of T-cytoplasm. The action of the gene Rf3, the single gene which restores fertility to the USDA cytoplasm, differs from that of Rf1 and Rf2 in that its effect is gametophytic rather than sporophytic (Buchert, 1961). Fifty per cent of the pollen from a plant heterozygous for Rf3 develops normally (those pollen grains that carry the dominant form of the gene). This is in contrast to the complete sterility of plants carrying Texas cytoplasm and heterozygous for Rf1 and Rf2. Duvick (1965) notes that there is variation in the level of fertility between full fertility and full sterility which would suggest the presence of modifying genes or multiple alleles of the Rf3 locus.

Duvick (1965) classified 32 sources of cytoplasmic male-sterility by their fertility restoration requirements using three tester inbreds. Twenty-five had the same reaction with the three tester lines as USDA cytoplasm and five had the same reaction with the testers as Texas cytoplasm. The other two sources appeared to be different from either USDA or Texas cytoplasm. Duvick interpreted these results as evidence that many different sources of sterilizing cytoplasm are identical. Beckett (1971) carried out a similar study with 30 sources of sterilizing cytoplasm and 18 tester inbreds. He concluded that there existed three types of sterilizing cytoplasm, Texas, USDA and Charrua (C). Two of the cytoplasms he tested fell into this latter group. A study by Gracen and Grogan (1974) using 38 sources of sterilizing cytoplasm and 28 tester inbreds also suggested the existence of three types of cytoplasm. Five of the 38 cytoplasms could not be classified into these three groups. The genetics of fertility restoration to this third group (C) has not yet been studied (Gracen and Grogan, l.c.).

Duvick (1965) had concluded that both T and S male-sterile cytoplasms were "notoriously" stable. Singh and Laughnan (1972), however, reported an apparent instability of the S cytoplasm. In progeny from the cross of a previously stable sterile and its maintainer, a number of male-fertile and partially male-fertile plants were obtained. Analyses of these fertile plants through test-crosses to male-sterile plants and observation of F2, F3 and F4 progeny indicated that the exceptional male-fertility could not be attributed to changes in the nucleus. It was proposed that a change had occurred in the cytoplasm, from male-sterile to normal-type cytoplasm. Other instances of apparent change to non-sterilizing cytoplasm were later detected, but only in

certain genotypes (Laughnan and Gabay, 1973). Laughnan and Gabay (l.c.) reported the discovery of certain lines, derived from genotypes which had exhibited cytoplasmic instability, in which reversion to fertility was transmissible through the pollen. These changes in the nucleus could be accounted for as formal mutations at restorer gene loci. Laughnan and Gabay proposed that as these changes occurred in the same strains which exhibited cytoplasmic instability, then the two events had a common basis. It was suggested that the initial appearance of the male-fertile element in the sterile cytoplasm, was followed by incorporation into the nucleus. This element was thus comparable to a bacterial episome.

Further evidence giving support to the possible involvement of episomes in restoration was cited by Laughnan and Gabay (1973). Plants carrying the USDA cytoplasm and heterozygous for the restorer gene (Rf3 rf3) normally produce 50% viable pollen grains carrying the Rf3 allele, while pollen grains carrying the rf3 allele are inviable. Laughnan and Gabay (l.c.), noted Duvick (1965) had called attention to the fact that lines heterozygous for Rf3 produce a small percentage of viable pollen grains with the genotype rf3. They suggested these occasional pollen grains with apparent genotype rf3 were originally Rf3, but had lost the Rf3 allele from the nucleus as an episome during the development of the male gametophyte.

Laughnan and Gabay (1975) attempted to identify the chromosomal location of the nuclear factors for fertility that developed in sterile lines. Tests of a number of lines carrying the new restoration genes revealed that they were not allelic with the restoration gene Rf3; furthermore, in the six lines tested the genes were not allelic with

each other. Studies of linkage groups also suggested that the newly arisen restorer genes occupied different chromosomal sites in different lines. This was taken as further evidence of the episomal nature of this factor, a characteristic of episomes in bacterial systems being their ability to transpose from one chromosomal site to another.

Sorghum. Cytoplasmic male-sterility in sorghum (Sorghum bicolor L. Moench) was first reported by Stephens and Holland (1954). The sterility resulted from the interaction of milo (race Durra) cytoplasm with nuclear factors from Kaffir races. More than two genes were thought to be involved in restoration. Maunder and Pickett (1959) obtained a one fertile:one sterile ratio in ten of eleven testcross progeny supporting a hypothesis for control of restoration by one dominant nuclear gene. F_2 data also supported this hypothesis, although restoration was incomplete in fertile plants. Kidd (1961), using two sources of restorer genes, concluded that three dominant modifying genes could bring about complete restoration in addition to the major restorer gene. Pi and Wu (1961) also reported that restoration was controlled by a single gene pair in some crosses but by two gene pairs in others. Miller and Pickett (1964) investigated partial male-fertility in sterile restorer hybrids and concluded that inter- and intra-allelic reactions in the genotype $Pf_1Pf_1Pf_2PF_2$ resulted in 100% fertility, while the fertility of $PF_1PF_1PF_2PF_2$ was lowered to 90% and that of $PF_1PF_1pf_2pf_2$ and $pf_1pf_1PF_2PF_2$ was lowered to 70% and 50% respectively.

Craigmiles and co-workers successfully transferred the cytoplasmic male-sterility of grain sorghum to sudangrass (S. arundinaceum Desv.

Stapf var. sudanese Stapf Hitch.). Craigmiles et al. (1958) reported that the cytoplasmic male-sterility had been transferred to Rhodesian sudangrass which had been found not to carry restorer genes. Craigmiles (1961) tested 13 other sudangrass lines and all showed 100% fertile progeny in both F1 and F2 suggesting that restoration was controlled by many genes. In further tests of these lines (Craigmiles, 1962) partial sterility was detected in the F2 and some completely sterile plants were found in testcross progeny. These results led Craigmiles to suggest that in four lines two gene pairs were controlling restoration and in two other lines three gene pairs were controlling restoration. Progenies of other lines could not be explained by major-gene action. Alam and Sandal (1967) in a similar study of six sudangrass lines concluded that restoration was conditioned by one gene pair in three crosses, two gene pairs in two crosses, and three gene pairs in one cross. In the latter case full fertility occurred when dominant genes were present at any two of the three loci for fertility restoration.

Millet. Burton (1958) first described cytoplasmic male-sterility in pearl millet (Pennisetum typhoides Burm. f. Stapf and Hubbard). The sterility first appeared in an inbred line and was stabilized through continued backcrossing of this line to another inbred. The cytoplasmic male-sterile line was designated Tift 23A. Two other sources of cytoplasmic male-sterility in pearl millet were discovered by Athwal (1961) in outcrossing populations. Burton and Athwal (1967) investigated the relationship of Tift 23A to these new sources (L66A and L67A). The latter were different from Tift. Although the maintainers for

these new sources also maintained the sterility of Tift 23A, the maintainer of Tift 23A was a fertility restorer for both L66A and L67A. Restorers for Tift 23A were found to be good maintainers for L66A and L67A. The maintainer of L66A was found to restore fertility to L67A. On the basis of these results Burton and Athwal (l.c.) suggested that each sterility source possessed a different sterilizing cytoplasm, and that each required a different restorer gene. Environment and modifying genes were suggested as influencing the action of restorer genes.

Burton (1972) reported the occurrence of heads with fertile sectors on cytoplasmic male-sterile plants. Analysis of seed from these fertile sectors revealed that in certain cases the phenomenon could be explained on the basis of mutation of a restorer gene from its recessive to its dominant form. In other cases, however, the possibility of mutation of the cytoplasm from sterilizing to normal could not be ruled out. Clement (1975) reported further on this reversion to fertility. He investigated four cytoplasmic male-sterile stocks which had previously shown reversion; three of these were derived from Tift 23A, the fourth was of African origin. The rates of appearance of fertile sectors were 0.03 heads per 100 for the African line and 0.15, 0.25 and 1.17 heads per 100 for the other three lines. In all cases analysis of self and cross progeny revealed the reversion to be cytoplasmic in nature and it was attributed to plasmon mutation. Rate of mutation appeared to be dependent on genotype. A further study by Burton (1977) found that 103 of 107 fertile mutants of Tift 23A behaved as maintainers also suggesting that their fertility was due to plasmon mutation. The remaining four mutants carried

mutations at the restorer gene locus.

Rice. Cytoplasmic male-sterility in rice (Oryza sativa L.) was first demonstrated by Shinjyo and Omura (1966), and further investigated by Shinjyo (1969). The sterility originated upon continued backcrossing of a cultivar belonging to sub-species indica with pollen from a cultivar of sub-species japonica. Genetic analysis by Shinjyo (l.c.) suggested that restoration was determined by a single dominant gene and was of the gametophytic type. Shinjyo (1972) later documented five sources of cytoplasmic male-sterility in rice, although restorers for only one of these was available.

Barley. Schooler (1967) obtained a cytoplasmic male-sterile form of the species Hordeum bulbosum L. by backcrossing a diploid H. vulgare L. x H. bulbosum hybrid as pollen parent with material having the cytoplasm of a wild Hordeum species (H. jubatum L.). The cytoplasmic male-sterile line was crossed with three barley cultivars, one of which appeared to carry restorer genes for the sterility. Schooler did not report further on this material, however, Ahokas (1978) has recently reported the production of cytoplasmic male-sterile barley by backcrossing H. vulgare as pollen parent with an Israeli strain of wild barley H. spontaneum Koch. A dominant gene for restoration was present in the wild species and about 15% of Finnish cultivars tested were partial restorers.

Ryegrass. Cytoplasmic male-sterility in ryegrass (Lolium sp.) was first reported by Nitsche (1971). The male-sterility was detected in a single plant of Italian ryegrass (L. multiflorum L.). Although its

cytoplasmic nature was established by backcrossing to lines of L. multiflorum, maintainer lines could not be developed. However, maintainer lines were developed from crosses with perrenial ryegrass (L. perrene L.). Wit (1974) also developed cytoplasmic male-sterile ryegrass. This line originated from a cross of a diploid L. multiflorum x L. perrene hybrid with autotetraploid Festuca pratensis L. Restoration of this sterility appeared to be determined by one or two dominant genes.

The Development of Pollen in Cytoplasmic Male-Sterile Plants

Wheat. Joppa et al. (1966) studied pollen and anther development in plants having the sterilizing cytoplasm of Triticum timopheevi. Florets of male-sterile plants contained shrivelled, sickle-shaped anthers at flowering. Meiosis was normal in the male-sterile plants; differences between sterile and fertile first appeared following the formation of exine and intine walls of the male gametophyte. In male-sterile plants the degeneration of the tapetum that normally occurs after first pollen grain mitosis was delayed and the tapetum persisted through to flowering. In fertile plants the tapetum had degenerated to a thin tissue lining the anther cavity at flowering. Pollen grains of the male-sterile were devoid of the starch granules that normally fill the grain and did not always contain the usual three nuclei.

Chauhan and Singh (1966) studied pollen abortion in a wheat carrying the sterilizing cytoplasm of Aegilops ovata. They recognized three types of abnormal development in anthers of sterile plants. In type (a) the tapetum degenerated prematurely followed by degeneration

of the sporogenous cells. In type (b) the degeneration of the tapetum was delayed and in type (c) the tapetum formed a periplasmodium which invaded the sporogenous cells. Only type (b) produced pollen grains but these were devoid of cytoplasm, nuclei and germination pore. Rai and Stoskopf (1974) also identified three types of tapetal behavior in lines with timopheevi cytoplasm. In one type abnormal growth and vacuolization of the tapetum prior to meiosis resulted in degeneration of the pollen mother cell prior to meiotic prophase. In a second type the tapetal cell walls broke down during meiosis arresting the development of the pollen mother cells which degenerated. Degeneration of the pollen mother cells did not occur where an adjacent sector of the tapetum remained intact. The third type of tapetal behavior which was observed in a majority of anthers involved the persistence of the tapetum through to flowering. All three types of tapetal behavior resulted in the production of pollen grains consisting of exine and intine with a germ-pore, two (occasionally three) nuclei, but devoid of starch grains.

Joppa et al. (1966) utilized transverse sections to observe the vascular system in anthers of fertile and sterile plants. The vascular bundles of sterile anthers were poorly differentiated and it was suggested that this was ultimately responsible for the abnormal development of pollen grains. Rao and Jain (1975) observed xylem development in anthers of plants from a population segregating for sterility. They obtained a highly significant correlation between the number of florets per head having anthers with normal xylem tissue and the seed set of

that head. They suggested that a block in the transport mechanisms of water and mineral salts was a factor in the abortion of pollen grains in male-sterile lines. Russian workers have also related sterility to abnormalities in the development of the anther vascular system (Protasevich and Palilova, 1975; Rjabinina, 1976). Rai and Stoskopf (1974), however, could find no evidence of abnormal differentiation of vascular bundles in either anther filament or anther connective tissue of sterile plants.

De Vries and Ie (1970) performed an electron-microscope study on anthers and pollen of cytoplasmic male-sterile wheat in an attempt to detect differences in the cytoplasmic structures of sterile and fertile lines. No differences could be detected in the cytoplasm of the tapeta of the two lines. Differences were found in the cytoplasm of pollen grains; the cytoplasm of sterile lines appeared degenerate and had few organelles in comparison to fertile lines. Turbin et al. (1974) compared the fine structure of the cytoplasms of pollen mother cells of sterile and fertile lines. They found fewer organelles in the cytoplasm and an increase in the vacuolization of the cytoplasm of pollen mother cells of sterile plants.

Corn. Rhoades (1933) carried out light-microscope studies on the Peruvian source of cytoplasmic male-sterility, the first sterilizing cytoplasm isolated. He noted that the cytoplasmic male-sterile types underwent a normal microsporogenesis. He also noted that the cytoplasm of pollen mother cells of sterile forms contained inclusions different in shape to those in normal cytoplasm. These differences were thought to be a consequence rather than a cause of male-sterility.

Degeneration of the microspores in the male-sterile line began around first pollen grain mitosis. Later investigations of the two sterilizing cytoplasms, Texas and USDA, revealed no differences in the cytoplasmic inclusions of sterile and fertile forms (Chang, 1954; Edwardson, 1955; Khoo and Stinson, 1957). In both types of cytoplasm meiosis was normal and degeneration of microspores began at first pollen grain mitosis as in the Peruvian type. Chang (1954) reported that the tapetal cells of lines carrying either Texas or USDA cytoplasm showed hypertrophy and persistence. Khoo and Stinson (1957) found that pollen grains of sterile lines were arrested in their development shortly after meiosis, wall thickening of the grains was limited, cell contents were granular and germ-pore development was faint. At anthesis pollen grains were thin-walled with a shrunken cytoplasm and contained no detectable starch.

Using the electron-microscope Edwardson (1962) investigated cytoplasmic inclusions in root-tip and tapetal cells of sterile and fertile plants. Whereas root-tip cells of sterile plants contained small unidentified inclusions (60μm diam.) in areas of dense cytoplasm, similar cells of fertile plants possessed neither inclusions nor dense cytoplasm. Similar inclusions were detected in tapetal cells of both sterile and fertile plants, however, those in sterile plants were slightly larger than those in fertile plants (58-64μm vs. 46-52μm). The inclusions in fertile plants were membrane-bound whereas those in sterile plants generally were not. The inclusions in both root-tip cells and tapetal cells appeared to contain RNA. Ryan and Ting (1974), however, could not find abnormal inclusions in root-tips of sterile

plants.

Palilova et al. (1967) investigated the ovular cytoplasm of sterile and fertile lines. The cytoplasm of ovules from sterile plants was strongly vacuolized, had a weakly developed endoplasmic reticulum and smaller starch grains than that of fertile plants. Mitochondria of sterile plants differed from those of fertiles in that they tended to be elongated and swollen with weakly expressed cisternae and a vacuolized matrix. In a later study (Turbin et al., 1968) it was shown that restoration of fertility was accompanied by restoration of the normal structure of the cytoplasmic components, in particular the mitochondria.

Sorghum. Maunder and Picket (1959) were the first to inquire into the events leading to pollen abortion in cytoplasmic male-sterile sorghum. Meiosis appeared to be normal, but pollen grains shrivelled shortly after they were formed. Singh and Hadley (1961) also reported that meiosis was normal, however, the cytoplasm of post-meiotic tapetal cells stained lighter with haematoxylin than comparable cells of a fertile line. Whereas the tapetal cells of a fertile line were binucleate and eventually disintegrated, those of the sterile line underwent endomitosis to become multinucleate and persisted during the development of the microspores. Damon (1961) studied a number of fertile and five cytoplasmic male-sterile sorghums. Three of the five sterile lines exhibited meiotic abnormalities, however, it was concluded that this was an inherited condition as the three lines were related to a common parent which also exhibited this abnormality. Although not responsible for sterility, the condition was exacerbated

when it occurred in conjunction with male-sterility. Young pollen of the two sterile lines which underwent a normal meiosis appeared almost normal but stuck together. At a later stage the pollen grains appeared to have broken down and clung together in masses.

Brooks et al. (1966) observed the tapetum of fertile and sterile lines. From prophase until the post-tetrad stage the radial width of tapetal cells remained constant in sterile, maintainer and restorer. However, after this stage the tapeta of maintainer and restorer gradually degenerated whereas that of sterile plants increased in width. In a small percentage of sterile plants a periplasmodial type of tapetum was observed. Microspores of the sterile line possessed an intine and exine but failed to undergo first pollen grain mitosis and did not accumulate starch.

Warmke and Overman (1972) investigated the behavior of callose in fertile and cytoplasmic male-sterile lines. Callose was found to be present initially as a thin layer surrounding each of the sporogenous cells. In fertile plants, as the anther and sporocytes enlarged, a central core of callose was developed. As enlargement continued the sporocytes separated from each other to line the inner face of the tapetum and each carried with it a small sector of the callose core. As meiosis progressed the callose became redistributed around the meiocyte. In a sterile anther the central callose failed to separate into sectors and instead formed an amorphous mass occupying the centre of the anther locule. By tetrad stage this callose had completely disintegrated whereas in fertile lines a thin layer of callose was present around each sporocyte liberated from the tetrad. As a result

of the abnormal behavior of callose the microspores of sterile anthers frequently stuck together and failed to maintain contact with the tapetum. The abnormal behavior of the callose was attributed to premature callose activity as had been documented by Frankel et al. (1969) in Petunia.

Overman and Warmke (1972) carried out an intensive investigation of the tapetal cells of fertile and cytoplasmic male-sterile sorghum. The tapeta of fertile and sterile did not differ until after meiosis, after the exine had been deposited onto the microspores. The tapetum of a fertile anther then became thinner, more dense and separation between individual tapetal cells became less clear. In sterile anthers the tapetum thickened and the cytoplasm became vacuolate. Whereas shrinking tapetal cells of fertile anthers retained their nuclei and non-vacuolate cytoplasm, tapetal cells of sterile plants lost much of their cytoplasm. In some cases radial tapetal walls ruptured to form an intratapetal syncytium. The tapeta of sterile anthers also differed in having a persistent inner tapetal wall, and a number of abnormalities not seen in fertile anthers. Overman and Warmke (l.c.) were unable to relate the abnormal tapetal behavior of steriles to their previous observations of abnormal callose behavior.

Rice. Shinjyo (1969) reported that pollen of cytoplasmic male-sterile plants was smaller and stained less deeply than normal pollen. Chu et al. (1972) reported that meiosis was normal in the cytoplasmic male-sterile, however, at the uninucleate stage about 25% of microspores of sterile plants had smaller nuclei. Microspores did not undergo the first pollen grain mitosis and possessed only one nucleus

at flowering. Mature pollen grains of sterile plants did not accumulate starch. No histological abnormality could be detected in the tapetum.

Barley. The anthers of the cytoplasmic male-sterile form discovered by Schooler (1967) exhibited thicker wall layers than normal anthers. At flowering sterile anthers were non-dehiscent but contained shrivelled pollen grains; these did not contain starch. The sterile form developed by Ahokas (1978) exhibited disturbances in the endothecial and tapetal layers. The endothecium of the sterile line never developed the secondary wall thickenings characteristic of normal anthers and endothecial chloroplasts were swollen and degenerate. The tapetum was persistent and produced greater than normal amounts of sporopollenin. The mature anthers of the sterile line were non-dehiscent. They contained the shells of pollen grains (exine but no intine) immersed in sporopollenin.

Ryegrass. Meiosis in the cytoplasmic male-sterile line discovered by Nitsch (1971) was regular, however, the tapetum usually degenerated prematurely. At dehiscence the anther locule was either hollow or collapsed. Pollen grains were occasionally present and contained degenerated cytoplasm. Wit (1974) did not investigate pollen development, however, he reported that anthers were pale, flat and papery in appearance and devoid of pollen grains.

The Stability of Fertility Restoration

Wheat. Schmidt (1964) first reported that the level of fertility restoration to cytoplasmic male-steriles was affected by environment,

particularly light intensity and/or duration and temperature. Robertson and Curtis (1967) grew a number of test-cross families in both greenhouse and field and found that for certain families the ratio of fertile to sterile progeny varied between the two environments. Wilson (1968) suggested that environments could be classified as "shallow sterile", "intermediate sterile" or "deeply sterile" with respect to fertility restoration. Best restoration would occur in a "shallow sterile" environment which was typified by a long growing season and high temperatures. A "deeply sterile" environment would have a short growing season and cool temperatures. Temperature was suggested by Wilson (l.c.) as being the most prominent environmental factor affecting fertility restoration.

Yen et al. (1969) defined the optimum greenhouse conditions for full restoration as 21°C, high humidity and a 16 hr photoperiod. Johnson and Patterson (1973) investigated fertility restoration in a number of genotypes at two temperatures (15 and 22.5°C) and at two photoperiods (16 and 20 hrs). Best restoration generally occurred at 15°C with a 20 hr. photoperiod and poorest restoration occurred at 22.5°C with a 16 hr. photoperiod. However, certain genotypes deviated from this pattern.

Bahl and Maan (1973) and Talaat et al. (1973) carried out monosomic analyses of a number of restorers. Year, location (N. Dakota or Mexico) and genotype of the restored line affected restoration. Certain restorer genes could only be detected in the "shallow sterile" environment of Mexico while genes that inhibited fertility were best detected in the "deeply sterile" environment of N. Dakota.

Corn. Edwardson (1955) documented the occurrence of partially male-sterile plants in a number of progenies which exhibited only full fertility or full sterility when grown the next year. He interpreted this as an effect of the environment of the first year inducing modification of the sterile condition to partially sterile. Information from regional tests also suggested that environmental factors influenced the expression of the male-sterile character. Jones et al. (1957a) concluded that humidity, temperature and daylength affected the expression of restoration. Whereas one restorer gene was adequate for full restoration under favourable conditions, in a season of low rainfall and high temperatures two or more genes were necessary.

Blickenstaff et al. (1958) suggested that daylength was the factor that determined the good restoration exhibited in southern Florida during the winter compared to the eastern United States during the summer. However, Duvick (1965) stated that the important contribution of the Florida winter to good restoration was the cool weather at flowering. Duvick (l.c.) suggested that for both USDA and Texas cytoplasms the environment just prior to and during anthesis was the critical factor. Hot dry weather promoted increased sterility while cool humid weather promoted increased fertility.

THE TRANSFER OF A CYTOPLASMIC MALE-STERILITY SYSTEM TO SPRING RYE

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Abstract

Six inbred lines of spring rye (Secale cereale L.) and an open-pollinated cultivar of spring rye were crossed with a cytoplasmic male-sterile line of winter rye. The open-pollinated cultivar and all except one inbred line exhibited segregation for fertility restoration. Through further inbreeding sub-lines of certain inbreds were obtained which were homozygous for maintenance of sterility or for restoration of fertility. Environmental effects on the expression of fertility restoration were detected.

Introduction

Putt (1954) identified an inbred line of rye (Secale cereale L.) in which male-sterility appeared to be maternally inherited. This appears to have been the first documentation of cytoplasmic male-sterility in rye, however, no further work with this material was reported. Discovery of cytoplasmic male-sterility in a number of winter ryes has since been reported by Russian workers (Zdril'ko, 1969; Kobyljanskij, 1969) and by Geiger and Schnell (1970). The latter discovered cytoplasmic male-sterility in the progeny of an inbred line crossed as male with an Argentinian cultivar "Pampa". Maintainer and

restorer lines for this source of sterility were successfully developed. These lines with winter habit were obtained from Dr. H.H. Geiger. This paper documents an attempt to transfer this source of cytoplasmic male-sterility to a number of lines of spring rye in order to decrease generation time and hence facilitate testing of the system in Manitoba.

Materials and Methods

Single plants of six inbred lines of spring rye and six plants of the open-pollinated spring rye cultivar "Gazelle" (Table 1) were crossed with the winter cytoplasmic male-sterile line. The same plants were also selfed by enclosing spikes in glassine bags prior to anthesis. The F_1 generation of each cross was grown in the greenhouse and anthesis was observed (male-sterile plants had small, non-dehiscent anthers). The selfed progeny of each male parent were also grown in the greenhouse. Spikes of secondary tillers of sterile plants in the F_1 populations were enclosed in glassine bags. When receptive these were pollinated with selfed progeny of the original male parent plant. As five of the six inbred lines were not fully homozygous (as evidenced by segregation in the F_1) and as each plant of open-pollinated Gazelle could be expected to be highly heterozygous, plants in a selfed progeny of an original male parent plant would not necessarily be genetically identical. Consequently, a number of plants derived from the original male parent plant were crossed with male-sterile F_1 plants to sample this variation.

The progeny from these first backcrosses were grown in the greenhouse together with plants derived from the self-pollination of each plant used as a male in the backcrosses. If a high percentage of

plants in the first backcross generation were male sterile then further backcrosses were made using those male lines which appeared to be approaching homozygosity with respect to maintenance of the sterility. Backcrossing was continued until some maintainer lines for the sterility were identified. If a high percentage of plants in the first backcross generation were male-fertile then plants from lines which appeared to be approaching homozygosity with respect to restoration of the fertility were crossed with male-sterile plants produced by maintainer lines. These F_1's were grown, scored for anther dehiscence and further crosses were made using plants of the most desirable lines (lines which produced a high percentage of male-fertile plants). This procedure was continued until plants within a line could be identified as having attained homozygosity with respect to restorer genes.

Except where noted, all material was greenhouse grown at 20°C. Supplemental illumination was provided by fluorescent lights (120 microeinsteins m^{-2} sec^{-1}) to give a 16 hr photoperiod.

Results

All except one F_1 progeny from the initial crosses of winter male-sterile with spring fertiles consisted of both male-fertile and male-sterile plants. Male-sterile plants produced anthers which were considerably reduced in size and which were non-dehiscent. This condition pertained to all tillers of a sterile plant. Usually the distinction between fertility and sterility was clear-cut although in certain cases partial fertility of the spike occurred. Such spikes produced typically non-dehiscent anthers at the top and bottom; however, the anthers of florets in the central portion of the spike

were dehiscent to varying degrees. All tillers of such plants exhibited this characteristic to a varying extent.

Inbred Line 28-10

Thirty-five percent of the F_1 population from the cross of the male-sterile with line 28-10 were sterile (Fig. 1). Male-sterile plants were crossed with three plants derived by selfing 28-10 designated 28-10a, b and c. Testing of lines from two of these plants was discontinued. Male-sterile plants were crossed with plants of 28-10a. Progeny from these crosses were seeded in the greenhouse in August 1975 and again in October 1975. The progenies of two of the crosses did not contain any sterile plants (Table 2).

Inbred Line 95-6

The F_1 population of winter male-sterile crossed with line 95-6 consisted of almost equal numbers of fertile and sterile plants (Fig. 1). One sterile plant was crossed with a single plant from the selfing of 95-6 designated 95-6a. The progeny consisted of five fertile and 11 sterile plants. Sterile plants were further crossed with plants derived from the selfing of 95-6a. Partially fertile plants occurred in three of the five progenies. In view of this partial fertility and also the fact that the five inbred sub-lines were very weak no further testing was done with this material.

Inbred Line 192-1

Eighty percent of the F_1 population from the cross of winter male-sterile with line 192-1 were male sterile (Fig. 1). Two sterile plants were crossed with two plants from the selfing of 192-1. All plants

from the first cross were male-sterile and all except two plants of the second cross were male-sterile. Further study of the line 192-1b was discontinued in favour of 192-1a. Sterile plants were crossed with eight plants derived by selfing 192-1a. The fertility of these progenies is shown in Table 3. Due to space limitations the progenies had to be grown under two levels of supplemental illumination. Eight plants of each (with the exception of number 3) were grown under high light intensity (230 microeinsteins m^{-2} sec^{-1}) and the remainder were grown under the standard light intensity. Male-steriles were crossed with plants derived from 192-1a 2 to 5. Lines 1, 6, 7 and 8 were not included due to their low vigor. The progeny of these crosses were seeded in the greenhouse in the winter of 1975/76. Further crosses were made with some of this greenhouse-grown material. The progeny from these crosses and remnant seed from the greenhouse seeding were seeded in the field in the summer of 1976. Fertility data were taken in both environments (Table 4).

Inbred Line 194-1

The cross of the winter male-sterile with line 194-1 resulted in almost equal numbers of fertile and sterile plants (Fig. 1). Crossing of a male-sterile plant with a single plant derived from 194-1 (designated 194-1a) resulted in only sterile plants in the next generation. Five sterile plants were further crossed with five plants derived from 194-1a (194-1a 1 to 5). All except one progeny consisted of only sterile plants. Further crosses were made with a number of plants of each of lines 194-1a 1 to 4. These progenies were grown in the greenhouse in the winter of 1975/76; all were sterile except for

the progeny of a sub-line of 194-1a 2 in which one of 15 plants was fertile.

Inbred Line 363-5

Crossing the winter male-sterile with line 363-5 produced the only F_1 which did not segregate for fertility/sterility. All plants of the F_1 were fully fertile and only fertile progeny were obtained when male-steriles were crossed with the sub-lines developed from 363-5 through selfing. However, crosses of the male-sterile with a line that was later developed from reserve seed of an early inbred generation of 363-5 did give rise to some sterile progeny.

Inbred Line 372-5

Thirty-one percent of the F_1 progeny from the cross of line 372-5 with the winter male-sterile were sterile. Sterile plants were crossed to three plants of 372-5 designated 372-5a, b and c. Crosses with both 372-5a and 372-5b resulted in eight fertile and six sterile plants; the cross with 372-5c resulted in 16 fertile and two sterile plants. Further study of this material was discontinued as the vigor of lines derived from 372-5a, b and c was very low.

Gazelle

Six plants of Gazelle were crossed with the male-sterile. The six F_1 families were grown in the greenhouse in the spring of 1976. Due to the self-sterility of Gazelle, selfed seed was obtained from plants four and five only. One plant of each survived to maturity; two sterile plants were crossed with these. The progeny of sterile x Gazelle-4 consisted of 12 fertile and two sterile plants, that of

sterile x Gazelle-5 consisted of 12 fertile and six sterile plants. No selfed seed was obtained from Gazelle-5. Two selfed seeds were obtained from the single plant of Gazelle-4, but both produced weak plants; consequently, no further work was done with this material.

Discussion

Line 363-5 and sub-lines of 28-10 were identified as being homozygous for restoration of fertility to the cytoplasmic male-sterile. Homozygous maintainers of the sterility were isolated from sub-lines of 192-1 and 194-1. Of the six inbred lines initially crossed with the cytoplasmic male-sterile only one line (363-5) appeared to be homozygous with respect to restorer genes. The other five inbred lines gave rise to progeny which segregated for fertility-sterility indicating that although inbred, they had remained heterozygous at the loci responsible for restoration of fertility. These same six lines had previously been included in a yield test (Scoles and Evans, 1978) and line 363-5 was found to have the lowest vigor. This could also be indicative of the greater level of homozygosity of line 363-5. The lines had been inbred for either five or seven generations, by which time theory predicts that the level of homozygosity reached would be approximately 97% and 99%, respectively. If lines which theory predicts to be so highly homozygous show such segregation it would seem probable that there had been a greater than expected retention of heterozygous plants during inbreeding. Plants within a line having a higher level of heterozygosity would be expected to be the more vigorous plants and could have been unconsciously selected as the plants to use for further inbreeding.

Preservation of heterozygosity in spite of inbreeding has previously been reported in rye by Muntzing (1963). However, in this case it was found to be due to the superior seed setting ability of heterozygotes under certain environmental conditions. Preserved heterozygosity has also been reported in barley (Hordeum vulgare) by Jain and Allard (1960) where it was assumed that natural selection of heterozygotes with greater fitness than the corresponding homozygotes was occurring. In the present case preserved heterozygosity is thought to be a result of the tendency to select vigorous plants for selfing to provide seed for the next generation.

The results of further inbreeding of the inbred lines also suggest that the lines were not fully homozygous. During the isolation from segregating lines of sub-lines homozygous for maintenance or restoration of fertility the increased inbreeding often lead to a reduction in vigor such that further work with the line was not feasible. This fact led to the rejection of certain lines that might have been genetically desirable as either maintainers or restorers. Similarly, attempts to isolate lines homozygous for maintenance or restoration of fertility from the cultivar Gazelle failed due to the self-sterility of plants selected for selfing as well as the low vigor of plants from any selfed seed that was obtained. If cytoplasmic male-sterility is to be transferred to other lines it would seem advisable that only vigorous, highly inbred lines be used so that these problems can be avoided.

When the progeny of sub-lines of 192-1a crossed to male-steriles were grown in the greenhouse under each of two light intensities a

significant effect of light intensity on the expression of fertility or sterility was observed (X^2 => 400, $p<0.001$). Under the higher light intensity an increase occurred in the number of fertile segregants from 12.3% to 35.2% (Table 3). An environmental effect was also indicated when progeny of sub-lines of 192-1a crossed to the male-sterile were grown in either the greenhouse or the field. In the greenhouse, certain progeny exhibited partial fertility. When this same material or further crosses of this material were grown in the field there was no evidence of partial fertility (Table 4). Ignoring the partially fertile plants that occurred in the greenhouse grown material a significantly greater percentage of fertile plants occurred in the greenhouse (14.8%) compared to the field (4.6%) (X^2 = >1500, $P<0.001$). Again there is an indication of an environmental effect on the expression of fertility.

These results suggest that fertility tends to be displayed under more favourable growing conditions, i.e., greenhouse compared to field or high light intensity compared to low light intensity. This seems to be in agreement with the known environmental effects on cytoplasmic male-sterility in wheat (Triticum aestivum). The more favourable environment of Mexico was regarded as a "shallow sterile" environment by Talaat et al. (1973) in which restorer genes were expressed that could not be detected in the "deeply sterile" environment of North Dakota. Similarly, in corn (Zea mays) Jones et al. (1957) concluded that whereas one restorer gene was adequate for restoration under favourable conditions, in a season of low rainfall and high temperatures two or more genes were necessary. In the future, it would seem desirable to conduct studies of cytoplasmic male-sterility in rye under

the most favourable conditions available to ensure the full expression of restorer genes present in the material.

Further studies are now underway utilizing material synthesized in this study to determine environmental effects on restoration. The same material is also being used in genetic studies of restoration, and in a histological study of the sterility.

TABLE 1. Level of inbreeding and origin of the spring-rye material

Line number or cultivar	Number of generations inbred prior to experiment	Cultivar derived from
28-10	5	Minusinskaya
95-6	5	Marco-Juarez
192-1	5	Petka (Petkus)
194-1	5	O.J. Rye
363-5	7	Prolific
372-5	7	Prolific
Gazelle	0	---

TABLE 2. Segregation in the progenies of cytoplasmic male-sterile x 28-10a, grown on two occasions

	Date seeded in greenhouse			
	August 1975		October 1975	
Sterile x plant #	Fertile plants	Sterile plants	Fertile plants	Sterile plants
1	5	2	13	2
2	5	0	10	0
3	5	0	15	0
4	3	1	7	7
5	1	3	6	9
6	4	1	8	7
7	7	0	14	1
8	3	5	6	6
	33	12	79	32

TABLE 3. Segregation in the progenies of cytoplasmic male-sterile x 192-1a, grown under two intensities of supplemental light

Supplemental light intensity				
	High		Low	
Sterile x plant #	Fertile plants	Sterile plants	Fertile plants	Sterile plants
1	3	5	4	10
2	2	3	6	23
3	-	-	1	14
4	3	5	2	21
5	2	5	1	23
6	3	5	0	10
7	3	5	0	10
8	3	5	3	10

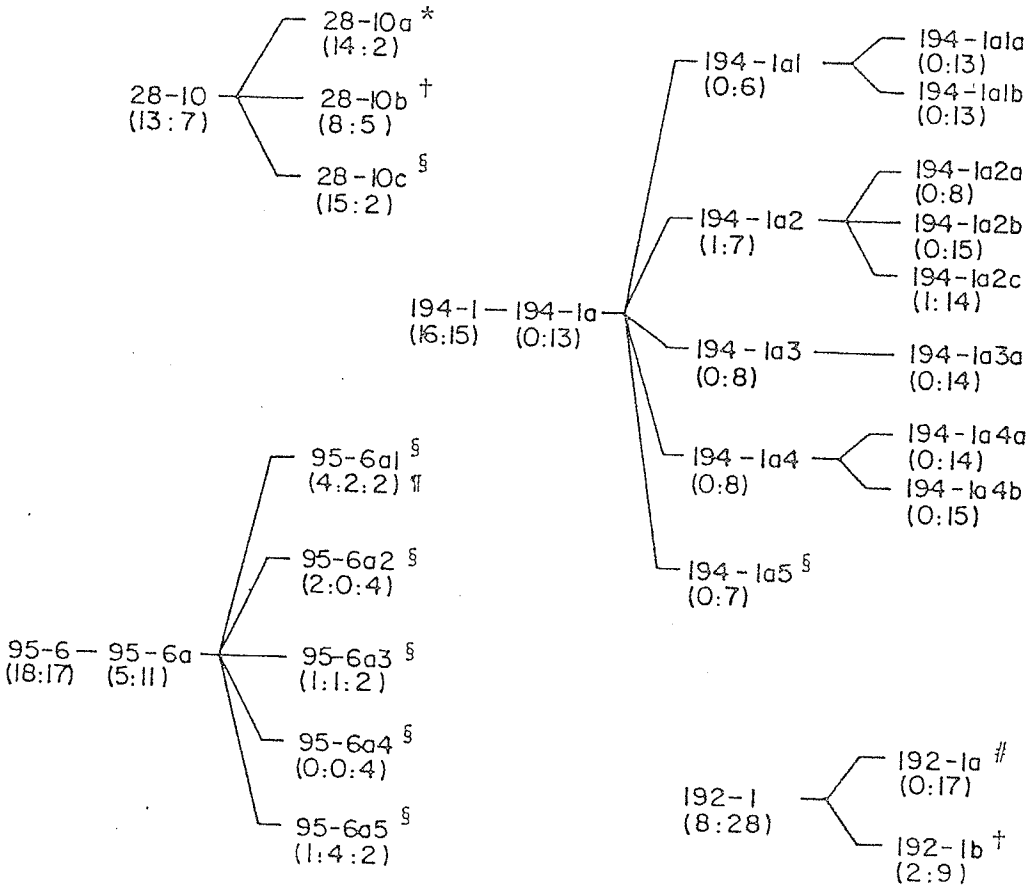
TABLE 4. Segregation in the progenies of cytoplasmic male-sterile x sub-lines of 192-1a 2, 3, 4, and 5

	Greenhouse 1975-76			Field 1976	
Sterile x plant #	Fertile plants	Partially fertile plants	Sterile plants	Fertile plants	Sterile plants
2-a	3	2	6	4	22
b	3	0	3	1	9
c	0	0	14	0	20*
d	0	0	14		
e	0	0	14	0	17*
f				4	15
g				0	13
h				1	9
i				1	17
j				0	9
k				0	7
l				2	7
3-a				0	16
b				3	11
c				3	18
d				1	11
e				0	12
4-a	3	3	6		
b	1	2	9	0	20*
c	5	3	5	3	16*
d	5	0	2		
e	0	0	13	0	18*
f	2	0	10	0	20*
g				0	16
h				0	16
i				0	9
j				0	17
k				0	17
l				0	13
5-a	3	0	7	3	15*
b	0	0	13	0	17*
c	0	0	15	0	20*
d	0	0	6	0	17*
e	0	0	7	0	18*
f				0	16
g				0	16
h				0	16
i				0	16
j				0	9

*indicates progeny from crosses made in the greenhouse in the winter of 1975-76

Figure 1. Derivation through inbreeding of the lines produced and the ratio of fertile to sterile progeny (bracketed) obtained when each line was crossed with the cytoplasmic male-sterile.

*see Table 2 for results of 28-10a x cytoplasmic male-sterile
†discontinued in favor of another line
¶indicates the ratio of fertile, partially fertile and sterile progeny
§discontinued due to low vigor of the inbred
#see Tables 3 and 4 for results of 192-1a x cytoplasmic male-sterile


28-10
(13:7)
28-10a *
(14:2)
28-10b †
(8:5)
28-10c §
(15:2)
95-6
(18:17)
95-6a
(5:11)
95-6a1 §
(4:2:2) ¶
95-6a2 §
(2:0:4)
95-6a3 §
(1:1:2)
95-6a4 §
(0:0:4)
95-6a5 §
(1:4:2)
194-1
(16:15)
194-1a
(0:13)
194-1a1
(0:6)
194-1a1a
(0:13)
194-1a1b
(0:13)
194-1a2
(1:7)
194-1a2a
(0:8)
194-1a2b
(0:15)
194-1a2c
(1:14)
194-1a3
(0:8)
194-1a3a
(0:14)
194-1a4
(0:8)
194-1a4a
(0:14)
194-1a4b
(0:15)
194-1a5 §
(0:7)
192-1
(8:28)
192-1a #
(0:17)
192-1b †
(2:9)

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THE EFFECT OF TEMPERATURE ON POLLEN FERTILITY AND ANTHER DEHISCENCE OF CYTOPLASMIC MALE-STERILE RYE

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THE EFFECT OF TEMPERATURE ON POLLEN FERTILITY AND ANTHER DEHISCENCE OF CYTOPLASMIC MALE-STERILE RYE

Abstract

Pollen fertility and anther dehiscence of two cytoplasmic male-sterile lines of spring rye (Secale cereale L.), their maintainers, their restorers and the F_1 between each sterile and restorer were investigated at three temperature regimes (15/10, 20/15 and 25/20°C). In a second experiment, the anther dehiscence of five additional sterile-restorer hybrids was investigated at the same temperature. Anthers of male-sterile plants did not contain pollen grains and were non-dehiscent at all temperatures. Pollen fertility of maintainer, restorer and sterile/restorer hybrids varied with temperature. All anthers of maintainer and restorer lines were fully dehiscent, but partially dehiscent and non-dehiscent anthers occurred in the sterile/restorer hybrids. Anthers of florets in the upper and lower portions of spikes of the sterile/restorer hybrids were often partially dehiscent or non-dehiscent. Variation among tillers of a plant with respect to this character was low, but variation among plants of a sterile/restorer hybrid was high suggesting genetic segregation. The classification of an anther as either dehiscent, partially dehiscent or non-dehiscent was directly related to pollen fertility. Better restoration of fertility was obtained at temperatures of 20/15 or

25/20°C than at 15/10°C.

Introduction

Reports of temperature affecting the sterility of cytoplasmic male-sterile plants are rare. Van der Meer and van Bennikom (1969) and Marshall et al. (1974) have reported the only temperature dependent occurrences of partial fertility in cytoplasmic male-sterile lines of onion (Allium cepa) and cotton (Gossypium hirsutum) respectively.

In contrast to the apparent stability of cytoplasmic male-sterility, effects of environment on fertility restoration of cytoplasmic male-steriles have been documented in at least eight species (Edwardson, 1970). Duvick (1965) concluded that temperature was the major environmental factor that affected pollen production of fertility restored cytoplasmic male-sterile corn (Zea mays). Similarly, Wilson (1968) reported that temperature was the most prominent variable affecting the expression of fertility restoration in wheat (Triticum aestivum). However, data as to the effects of temperature on fertility restoration are often incomplete and contradictory.

Geiger and Schnell (1970) discovered cytoplasmic male-sterility in rye (Secale cereale L.), and developed maintainer and restorer lines for this sterility. Scoles and Evans (1979) transferred this sterility from the original winter habit lines to lines of spring habit and identified other maintainer and restorer lines. The present study investigated the effect of temperature on anther dehiscence and pollen fertility of cytoplasmic male-sterile rye lines, of male-fertile maintainer and restorer lines and of sterile/restorer hybrids.

Materials and Methods

Plants of eight lines of rye, two lines of each of four genotypes (sterile x maintainer, sterile x restorer, maintainers and restorers) were utilized in the first experiment (Table 1). The inbred lines were developed by Scoles and Evans (1979) and had been inbred for eight generations. Seeds of the eight lines were germinated in the laboratory and 15 uniform seedlings of each were transplanted, one per pot, to 12 cm pots containing a 2:1:1 soil, sand, peat mix. Five plants of each line were assigned to one of three controlled environment cabinets. Plants of the eight lines within a cabinet were randomly arranged. Plants were exposed to a 16 hr photoperiod with light/dark temperatures of 15/10, 20/15 and 25/20^{o}C respectively (light intensity 250 micro-einsteins m^{-2} sec^{-1}). Just prior to anthesis a spikelet was taken from the central third of the spike of the two main tillers of each plant. Pollen fertility was evaluated in each floret of these spikelets by squashing one anther from each floret in a drop of IKI (Jensen, 1962). The number of starch-filled and empty pollen grains was scored using low magnification (X100) of a microscope.

Anthers remained attached by their filaments to the spike for a considerable period of time after anthesis and consequently they could be classified with respect to dehiscence type. Anthers from heads of the first three tillers of each of the eight lines were classified as fully dehiscent (the anther locules were split along their full length), partially dehiscent (locules were split for only a portion of their length) or non-dehiscent.

Because of the variation that occurred among sterile/restorer hybrid plants in the first experiment, a second experiment was

performed in which the progeny of five other sterile x restorer crosses were grown at the three temperature regimes. Anthers from the spike of the main tiller of each plant were classified as in the first experiment; the spike was then assigned a score according to the following scale:

1. all anthers non-dehiscent,
2. anthers in less than one-half of the head partially dehiscent,
3. anthers in less than one-half of the head fully dehiscent,
4. anthers in more than one-half but less than three-quarters of the head fully dehiscent,
5. anthers in more than three-quarters of the head fully dehiscent.

A mean value for each line at each temperature was then calculated.

In order to relate pollen fertility to the dehiscence pattern, two spikes of each of the five classes were selected and all spikelets were taken from one side of each spike just prior to dehiscence. These spikelets were fixed in Farmer's fluid (Jensen, 1962) and stored in a refrigerator. Pollen fertility was later analysed using one anther from each floret of all spikelets.

Analyses of variance were performed on the data of both experiments. Duncan's multiple range test (Snedecor and Cochran, 1967) was used to designate significantly different mean values ($P = 0.05$).

Results and Discussion

Pollen could not be found in anthers of either of the male-sterile lines at any temperature (Table 2), and all anthers were non-dehiscent. Only in cotton and in onion has temperature been reported to affect the expression of sterility in cytoplasmic male-sterile plants. In cotton,

day temperatures above 33°C were required for full sterility while temperatures less than 27°C resulted in almost full (96%) fertility (Marshall et al., 1974). In onion, Jones and Clarke (1943) found a greater than expected number of sterile plants in segregating progenies when they were grown in the greenhouse at very high temperatures (temperature not reported). On the contrary, Van der Meer and Van Bennikom (1969) found all plants in a population of onions were male-sterile at 14°C whereas at 30°C only 71% were male-sterile. In the latter two reports, the behavior of plants within both male-sterile and fertile maintainer lines was not uniform at a given temperature and a change in temperature altered the ratio of fertile to sterile plants in the lines. We suggest these lines had fertility-restoring genes whose expression was temperature dependent. The presence of such genes in wheat has been well documented (Bahl and Maan, 1973). The effect of temperature on the cytoplasmic male-sterility of cotton might also be explained by temperature dependent restorer genes, however, the possibility that the sterilizing cytoplasm itself was sensitive to temperature cannot be ruled out.

Analysis of variance of the pollen fertility of the six male-fertile lines used in this study indicated highly significant ($P = 0.01$) effects for lines and temperatures and a highly significant ($P = 0.01$) interaction. The pollen fertility of the two restorer lines was similar at each temperature regime (Table 2). The pollen fertility of the two maintainer lines was also similar at each temperature, but in contrast to the restorer lines, their fertility was highest at the intermediate temperature.

The mean pollen fertility of the two sterile/restorer hybrids

fluctuated over the three temperatures (Table 2). Anther dehiscence of these lines was variable, partially dehiscent and non-dehiscent anthers occurred in some spikes (Fig. 1). In contrast, all anthers of both maintainer and restorer lines were fully dehiscent at the three temperatures. As a consequence of the variation in anther dehiscence in sterile/restorer hybrids, pollen fertility figures obtained from one spikelet of a spike were highly dependent on the location of the spikelet sampled.

As all florets within a head were of the same genotype, then the observed variability must have been an environmental effect related to floret location within the head. Where such variation occurred, the level of dehiscence was with one exception greatest in the anthers of the central sector of the head. The exception was one plant in which all spikes possessed fully dehiscent anthers at the tip and base and partially dehiscent anthers in the central sector. In a rye spike, the first anthers to dehisce are those in the central sector. This suggests that the pattern of anther dehiscence observed in partially fertile rye spikes might be determined by anther age; the earlier anthers being more fertile than later ones.

Variation occurred among plants of sterile/restorer hybrids in the degree of fertility restoration (Fig. 1); this variation was greatest at the high temperature. At this temperature one plant of each sterile/restorer hybrid possessed only fully dehiscent anthers, while other plants possessed either a few partially dehiscent anthers (line 3) or only non-dehiscent anthers (line 7). Such variation was not evident at the intermediate and low temperatures, although plants of line 3 generally exhibited greater variation than plants of line 7

at both temperatures. At these lower temperatures there were no fully fertile plants comparable to those that occurred at the high temperature and no plants as sterile as those that occurred at the high temperature.

In contrast to the first experiment, variation among plants of the five sterile/restorer hybrids used in the second experiment was not restricted to the high temperature. Such variation occurred at all temperatures and in most lines (Fig. 2). That such variation in fertility should occur in F_1 hybrids would suggest that the restorer parent was not homozygous for genes governing restoration. However, during the development of the restorer line test-crosses with male-steriles revealed no such heterozygosity (Scoles and Evans, 1979). It is possible that some residual heterozygosity remained undetected or that some outcrossing occurred in the previous generation. The occurrence of this variation in the F_1 material makes it difficult to draw conclusions as to the effect of temperature on fertility restoration. The possibility of a treatment effect being the result of certain plants having been assigned to that treatment cannot be disregarded.

For the purposes of analysis of the experiment, the mean level of non-dehiscence was calculated for each plant of the sterile/restorer hybrids. The highly variable high temperature treatment was excluded from this analysis. The analysis indicated that at both intermediate and low temperatures line 3 possessed significantly ($P = 0.05$) larger non-dehiscent sectors than line 7, and that the lower temperature resulted in significantly ($P = 0.05$) larger non-dehiscent sectors for line 3 only. Analysis of the second experiment was performed on the

mean value of each sterile/restorer hybrid at each temperature (Table 3). Highly significant (P = 0.01) effects for both lines and temperatures were obtained. The level of dehiscence at the low temperature was significantly (P = 0.05) less than at the two higher temperatures. With one exception, the five sterile/restorer hybrids ranked in the same order at all three temperatures and lines 12 and 9 exhibited significantly (P = 0.05) less dehiscence than line 10.

Pollen fertility in non-dehiscent, partially dehiscent and fully dehiscent anthers of sterile/restorer hybrids was investigated in an attempt to relate anther dehiscence to the level of fertility. Anthers of the non-dehiscent class contained from 5-40% stainable pollen while partially dehiscent and fully dehiscent anthers contained from 40-60% and from 60-100% stainable pollen respectively. Thus, there was a direct relationship between the dehiscence of an anther and the amount of stainable pollen it contained. The non-dehiscent anthers that developed on plants of sterile/restorer hybrids differed from those of the male-sterile line in that they contained empty pollen grains, whereas the anthers of the male-sterile contained nothing identifiable as pollen.

The results of these two experiments lead us to suggest that as in other species, fertility restoration in cytoplasmic male-sterile rye is temperature-sensitive. Van Marrewijk (1969) carried out an extensive study of temperature effects on fertility restoration using cytoplasmic male-sterile Petunia (Petunia sp.). He concluded that in any species there was an optimum temperature for fertility restoration which was highly dependent upon genotype and environmental factors. Comparison of the results obtained in the present study with known

effects of temperature on fertility restoration in other species is difficult because of the complex relationship between fertility restoration, environmental factors and genotype. In wheat (Johnson and Patterson, 1973), in corn (Duvick, 1965) and in Petunia (van Marrewijk, 1969) the limited data available suggest that fertility restoration is more complete under cool conditions. In the present study, however, the poorest restoration occurred at the lowest temperature suggesting that the optimum temperature for fertility restoration in rye is higher than in some other species. This seems difficult to reconcile with the fact that rye is recognized as a cool-climate species. Further work in rye and in other species is needed to establish the relationship between temperature and fertility restoration. Hopefully this would lead to a better understanding of fertility restoration to cytoplasmic male-sterile species and allow greater use of cytoplasmic male-sterile systems.

TABLE 1. Genotype, line number and designated code of the material utilized in the two experiments

Genotype	Line number or hybrid	Code
	First Experiment	
Sterile	192-1a4m4	1
Maintainer	192-1a4m4	2
Restored sterile	192-1a4m4 x 28-10a2b	3
Restorer	28-10a2b	4
Sterile	192-1a4m1	5
Maintainer	192-1a4m1	6
Restored sterile	192-1a4m1 x 28-10a2e	7
Restorer	28-10a2e	8
	Second Experiment	
Restored sterile	192-1a4m12 x 28-10a2o	9
Restored sterile	192-1a4m9 x 28-10a2m	10
Restored sterile	192-1a4m11 x 28-10a2q	11
Restored sterile	192-1a4m4 x 28-10a2c	12
Restored sterile	192-1a4m12 x 28-10a2n	13

TABLE 2. The effect of temperature on the percentage of fertile pollen

Line #	Temperature (°C) 15/10	20/15	25/20
1	0	0	0
2	79.3* abc†	81.8 a	66.1 de
3	61.4 e	70.4 cde	37.5 g
4	71.7 abcde	72.1 abcde	74.9 abcd
5	0	0	0
6	73.6 abcd	82.9 a	70.2 cde
7	51.3 f	83.2 a	73.2 bcd
8	70.9 bcde	70.4 cde	73.7 abcd

*Each value represents the mean of two florets from each of two tillers from five plants

†Means of each character followed by the same letter are not significantly different

TABLE 3. The effect of temperature on the level of dehiscence of five sterile/restorer hybrids

Line #	Temperature (°C)			Overall mean
	15/10	20/15	25/20	
9	1.50*(26)†	3.09 (23)	2.60 (25)	2.37§bc
10	2.65 (11)	4.11 (9)	4.50 (9)	3.83 a
11	2.18 (17)	4.12 (17)	4.47 (17)	3.59 abc
12	1.15 (13)	2.00 (12)	1.50 (12)	1.55 c
13	1.75 (12)	3.69 (13)	3.91 (11)	3.12 abc
Overall mean	1.84§b	3.40 a	3.43 a	

*See materials and methods for method of calculating level of dehiscence

†The number of plants in a treatment

§Overall means within row or column followed by the same letter are not significantly different

Figure 1. The effect of temperature on the pattern of anther dehiscence along the spikes of the first three tillers of five plants of each sterile/restorer hybrid, ▮ = spikelet with fully dehiscent anthers, ‖ = spikelet with partially dehiscent anthers and | = spikelet with non-dehiscent anthers.

RESTORED STERILE #7

TILLER
PLANT

15/10°C

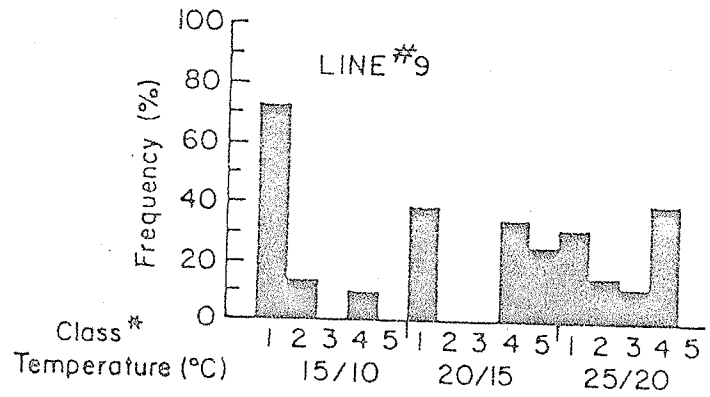
20/15°C

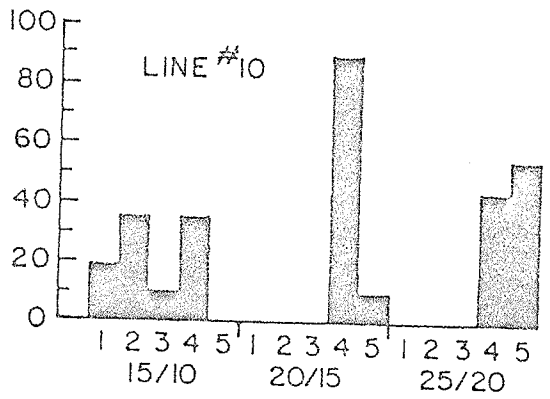
25/20°C

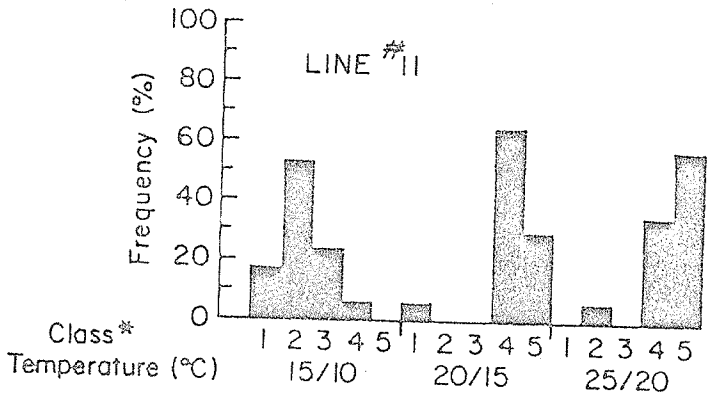
RESTORED STERILE #3

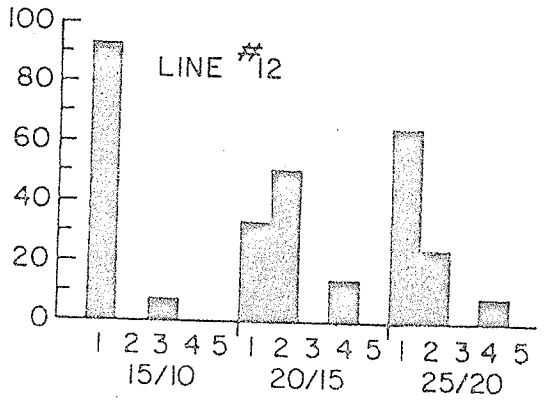
TILLER
PLANT

Figure 2. The effect of temperature on the pattern of anther dehiscence exhibited by five sterile/restorer hybrids, *see Materials and Methods for description of classes.


LINE #9
Frequency (%)
100
80
60
40
20
0
Class *
Temperature (°C)
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
15/10
20/15
25/20


LINE #10
100
80
60
40
20
0
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
15/10
20/15
25/20


LINE #11
Frequency (%)
100
80
60
40
20
0
Class *
Temperature (°C)
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
15/10
20/15
25/20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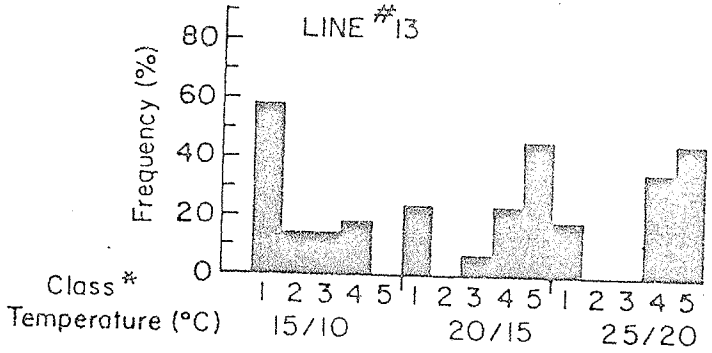
80
60
40
20
0
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
15/10
20/15
25/20


LINE #13
Frequency (%)
80
60
40
20
0
Class *
Temperature (°C)
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
15/10
20/15
25/20

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THE GENETICS OF FERTILITY RESTORATION IN CYTOPLASMIC MALE-STERILE RYE

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Abstract

Three inbred lines of rye (Secale cereale L.) known to be capable of restoring fertility to a cytoplasmic male-sterile line were crossed with the sterile line. The proportions of male-fertile, partially male-fertile and male-sterile plants in F_2 and backcross progenies indicated that three dominant restorer genes were present in each line. These were designated Rf_1, Rf_2 and Rf_3; their relative expressivity was $Rf_1 > Rf_2 > Rf_3$. Expressivity was dependent upon environment. Partial fertility occurred when certain genotypes carried two of the three alleles as dominant, but was dependent upon genotype and environment.

Introduction

If cytoplasmic male-sterile (CMS) systems are to be successfully utilized in the production of hybrid grain crops fertility restoration in the F_1 is essential. Sources of CMS and lines carrying restorer genes have now been discovered in a number of species. Knowledge of the number of genes involved in restoration is important in the development of good restorer lines.

In a majority of species, fertility restoration is of the sporophytic type and all pollen grains of plants heterozygous for the

restorer gene(s) are functional (Edwardson, 1970). However, in one type of CMS corn (Zea mays) and in rice (Oryza sativa), gametophytic restoration systems where only those pollen grains carrying the dominant form of the restorer gene are functional have been reported (Buchert, 1961; Shinjyo, 1969 respectively).

In corn, two dominant genes were found to restore fertility to CMS lines possessing Texas (T) cytoplasm (Duvick, 1959) while one gene restored fertility to CMS lines possessing S cytoplasm (Buchert, 1961). Modifier genes, essential for full fertility restoration to the T CMS form in all except the most favourable environments have also been detected (Edwardson, 1955; Beckett, 1966). In wheat (Triticum aestivum), dominant genes restoring fertility to lines carrying the timopheevi sterilizing cytoplasm have been detected on at least seven wheat chromosomes, although no more than three chromosomes carrying restorer genes have been detected in any one restorer line. A number of modifier genes have also been detected and up to six chromosomes have been found to carry modifier genes in some cultivars. Scoles (1979) has reviewed the available data on restorer genes for the timopheevi cytoplasm; only two of the 21 wheat chromosomes have not been implicated in fertility restoration as carrying either a restorer or modifier gene.

Cytoplasmic male-sterile systems have also been developed in other members of the Gramineae, including sorghum (Sorghum bicolor), sudangrass (Sorghum arundinaceum var. sudanense), millet (Pennisetum typhoides) and rice. Fertility restoration in sorghum was found to be determined by one dominant gene (Maunder and Pickett, 1959) although Kidd (1961) also detected three dominant modifier genes that were

necessary for complete restoration. In sudangrass full restoration was achieved by one, two or three genes (Alam and Sandal, 1967). One gene restored fertility in millet (Burton, 1972), however, Burton and Athwal (1967) suggested the occurrence of three genes and modifiers. In rice, Shinjyo (1969) could detect only one restorer gene of the gametophytic type.

Geiger and Schnell (1970) reported the development of CMS rye (Secale cereale L.) possessing the cytoplasm of an Argentinian cultivar, Pampa. Pampa appeared to carry restorer genes for this sterility and other inbred lines have since been found that carry restorer genes (Geiger, 1972; Geiger and Morgenstern,1975; Scoles and Evans, 1979) but the genetics of restoration has not been reported. Other sources of CMS in rye have been developed and in some cases the genetics of restoration has been analysed. Kobyljanskij (1969) and Klyuchko and Belousov (1972) reported that restoration was accomplished by a single dominant gene in some CMS lines although other lines were detected in which restoration involved more than one gene (Kobyljanskij, 1971; Kobyljanskij and Katerova, 1973). Polygenic control of restoration has also been reported (Katerova, 1975; Zdril'ko and Adamchuk, 1975). Lapinski (1972) developed CMS Secale montanum and S. kuprijanovii by backcrossing these species to S. cereale as the female. Single dominant restorer genes and also modifier genes were detected in some plants of S. montanum and S. kuprijanovii. In the present study F_2 and backcross data were analysed in an attempt to obtain a better understanding of fertility restoration to the Pampa CMS system.

Materials and Methods

Three unrelated inbred lines (Table 1) thought to be homozygous with respect to restorer genes were crossed with either spring or winter forms of CMS plants presumed to be homozygous recessive for restorer genes. Restorer lines 363-5 and 28-10 were developed by Scoles and Evans (1979) while line R32 was derived by inbreeding from line L-18-F developed by Geiger (1972).

In all crosses restoration was complete in every F_1 plant. Seed set on F_1 plants of the cross 363-5 x winter CMS was very poor; consequently, seed from 13 F_1 plants was bulked to give a single F_2 progeny. Single F_1 plants from each of the crosses 28-10a3a to f x spring CMS were selfed to give six F_2 progenies. The F_2 progenies from 363-5 x CMS and from lines 28-10a3a to f x CMS were grown in the greenhouse at 20°C and received supplemental illumination (120 microeinsteins m^{-2} sec^{-1}) for 16 hr. Ten F_1 plants from the cross R32 x spring CMS were selfed and seed from each F_1 plant was treated as a separate F_2 progeny; these 10 plants were also used as pollen parents in crosses with CMS plants to give 10 backcross progenies. Both F_2 and backcross progenies from R32 x CMS were grown in a growth room (20°C, 250 microeinsteins m^{-2} sec^{-1}, 16 hr photoperiod) and also in the field in the summer of 1978. F_2 progeny of 363-5 x winter CMS were not vernalized, but F_2 and backcross progenies of the winter line R32 x CMS were vernalized for 8 wk at 2°C before being transplanted into the growth room or field.

Anthers of plants of the F_2 and backcross progenies were observed at intervals during anthesis. When anthesis in all tillers of a plant was complete, the plant was classified as either fertile, partially

fertile, or sterile. Data were analysed by X^2 (Strickberger, 1976) to test for goodness of fit to expected genetic ratios.

Results and Discussion

Line 363-5 x CMS

The unvernalized F_2 progeny consisted of 227 plants; 168 were of spring habit, consistent with a 3:1 ratio ($X^2 = 0.12$, $p = 0.9 - 0.7$). These data agree with the finding of Purvis (1939) that spring habit in rye is determined by a single dominant gene. Seventy-eight of the 168 F_2 plants were fully fertile, 24 were partially fertile and 66 were sterile; these data fit a 27:9:28 ratio ($X^2 = 1.49$, $p = 0.5 - 0.25$). This ratio would be expected if restoration were dependent on three complementary dominant genes which we will designate Rf_1, Rf_2 and Rf_3. Fertile phenotypes would have the genotype $Rf_{1-}Rf_{2-}Rf_{3-}$; partially fertile genotypes would have the phenotype $Rf_{1-}Rf_{2-}rf_3rf_3$ and all other genotypes would be sterile.

Line 28-10 x CMS

The data of F_2 progenies from plants 28-10a3b, c, e and f (Table 2) fit a 27:9:28 ratio both individually and when pooled, thus supporting the data from 363-5 x CMS. However, progeny data from plants a and d (Table 2) deviated significantly from this ratio. Both of these progeny consisted of an increased number of partially fertile plants at the expense of both fertile and sterile types.

Line R32 x CMS

Data from the six F_2 progenies grown in the growth room (Table 3) did not fit a 27:9:28 ratio. However, data of five of the six progenies

gave a good fit to a 36:9:19 ratio. This ratio would be expected if restoration were dependent on three genes as before, but with different interactions to those suggested for lines 363-5 and 28-10. The previously partially fertile genotype $Rf_{1-}Rf_{2-}rf_3rf_3$ would now be fertile as would $Rf_{1-}Rf_{2-}Rf_{3-}$; $Rf_{1-}rf_2rf_2Rf_{3-}$ would be partially fertile, while $rf_1rf_1Rf_{2-}Rf_{3-}$ and all other genotypes would be sterile. By this hypothesis the backcross progeny would be expected to exhibit a 2:1:5 ratio. Data from five of the seven backcross progeny gave a good fit to this ratio (Table 4). When pooled, data from six of the seven backcross progeny also gave a good fit to this ratio.

When the 10 F_2 progenies from R32 x CMS were grown in the field, results were more variable than in the growth room (Table 5). However, five of the 10 sets of data did fit a 27:9:28 ratio and the summed data also gave a good fit to this ratio although the homogeneity X^2 was large and significant. If families from plants number two, three and eight were removed from the analysis then the homogeneity X^2 became non-significant. By this hypothesis the backcross progenies grown in the field would be expected to fit a 1:1:6 ratio rather than the 2:1:5 ratio found in the growth room. Four of the five backcross progenies grown in the field did fit this ratio (Table 6).

These results suggest that fertility restoration in this material is controlled by three genes. Partial fertility sometimes resulted when two of these three loci carried at least one dominant allele. However, the expression of partial fertility was dependent upon the particular loci involved and upon the environment. If only Rf_1 and Rf_2 were present as dominants, plants were fully fertile in the growth

room but partially fertile in the field or greenhouse. Plants were partially fertile in the growth room but sterile in either the field or greenhouse when only Rf_1 and Rf_3 were present as dominants. Sterility occurred in all genotypes with rf_1rf_1. This suggests that the three genes can be ranked in order of expressivity as $Rf_1>Rf_2>Rf_3$. The growth room appears to be a more favorable environment for expression of restoration than either the field or greenhouse. Differences in expressivity of restorer genes and environmental effects on expressivity have previously been documented in wheat (Talaat et al., 1973; Bahl and Maan, 1973) and in other species (Edwardson, 1970).

Not all of the present results could be explained by the above hypothesis. Two of the six F_2 progenies of 28-10a3 x CMS deviated significantly from the 27:9:28 ratio exhibited by the other four progenies. These two progenies exhibited almost identical ratios suggesting that the deviation was due to genetic rather than environmental causes. The data from these two progenies (a and d) were tested for goodness of fit to a 27:15:22 ratio. Both gave a good fit to this ratio (X^2 = 2.03, p = 0.5 - 0.25 and X^2 = 1.25, p = 0.75 - 0.5 respectively). This ratio would result if in these particular F_2 progenies the genotypes $Rf_1Rf_1rf_2rf_2Rf_3rf_3$ and $Rf_1rf_1rf_2rf_2Rf_3rf_3$ were partially fertile while $Rf_1Rf_1rf_2rf_2Rf_3Rf_3$ and $Rf_1rf_1rf_2rf_2Rf_3Rf_3$ were sterile (i.e. when Rf_1 and Rf_2 were dominant and recessive respectively; heterozygosity at the third allele resulted in partial fertility while homozygosity resulted in sterility). Intra-allelic interactions at the third locus are presumed to result in the greater fertility of the heterozygote over that of the homozygous dominant genotype. Miller and Picket (1964) have previously suggested the

occurrence of such heterosis at the restorer gene loci of sorghum. However, it is also possible that the deviations observed in the present study were due to segregation of additional restorer genes. The restorer lines, although highly inbred, may not have been fully homozygous for all restorer genes. Muntzing (1963) and Scoles and Evans (1979) have previously documented preserved heterozygosity in rye and it is possible that one or more restorer genes could still have been in the heterozygous condition resulting in other ratios. Deviation could also have been due to modifier genes present in either the restorer lines or the CMS line.

TABLE 1. The level of inbreeding, habit and origin of the inbred restorer lines used in crosses with CMS plants

Inbred line number	Number of generations inbred	Habit	Cultivar of origin
363-5	7	Spring	Prolific
28-10a3a to f	8	Spring	Minusinskaya
R32	20	Winter	Petkus

TABLE 2. The fertility and X^2 analysis of six F_2 progenies derived by crossing six plants of line 28-10a3 with a CMS line

Line Number	Fertile	Partially fertile	Sterile	d.f.	X^2 27:9:28	Probability
28-10a3b	29	9	33	2	0.25	0.9-0.75
c	37	12	21	2	5.39	0.1-0.05
e	31	12	29	2	0.50	0.9-0.75
f	88	43	101	2	4.30	0.25-0.1
Summed data	185	76	184	2	3.49	0.25-0.1
		Homogeneity		6	6.95	0.5-0.25
28-10a3a	50	36	52	2	16.5	0.005
d	25	19	26	2	9.92	0.01-0.005

TABLE 3. The fertility and X^2 analysis of F_2 progenies grown in the growth room derived from the cross of R32 with a CMS line

Line number	Fertile	Partially fertile	Sterile	d.f.	X^2 36:9:19	Probability
R32-1	35	8	16	2	0.24	0.9-0.75
2	35	5	11	2	3.18	0.25-0.1
3	35	8	15	2	0.46	0.9-0.75
4	35	6	13	2	1.61	0.5-0.25
5	27	0	9	2	7.58	0.025-0.01
8	28	9	19	2	1.26	0.75-0.5
Summed data	195	36	83	2	4.54	0.25-0.1
		Homogeneity		10	9.79	0.5-0.25

TABLE 4. The fertility and X^2 analysis of backcross progenies grown in the growth room derived from the cross of R32 with a CMS line

Line number	Fertile	Partially fertile	Sterile	d.f.	X^2 2:1:5	Probability
R32-1	13	8	33	2	0.27	0.9-0.75
2	15	9	74	2	7.20	0.05-0.025
3	11	7	30	2	0.25	0.9-0.75
4	11	10	22	2	4.86	0.1-0.05
5	15	5	45	2	1.77	0.5-0.25
10	8	8	25	2	2.12	0.5-0.25
Summed data	73	47	229	2	3.13	0.25-0.1
		Homogeneity		12	13.34	0.5-0.25
R32-9	19	5	8	2	22.58	0.005

TABLE 5. The fertility and X^2 analysis of F_2 progenies grown in the field derived from the cross of R32 with a CMS line

Line number	Fertile	Partially fertile	Sterile	d.f.	X^2 27:9:28	Probability
R32-1	14	5	15	2	0.02	0.99-0.975
4	15	13	25	2	6.70	0.05-0.025
5	24	10	22	2	0.88	0.75-0.5
6	20	4	26	2	2.14	0.5-0.25
7	28	12	28	2	0.74	0.75-0.5
9	25	11	13	2	6.68	0.05-0.025
10	33	5	34	2	3.02	0.25-0.1
Summed data	159	60	163	2	0.86	0.75-0.5
		Homogeneity		12	19.32	0.1-0.05
R32-2	3	6	5	2	9.89	0.01-0.005
3	55	11	28	2	10.50	0.01-0.005
8	2	0	17	2	16.28	0.005

TABLE 6. The fertility and X^2 analysis of backcross progenies grown in the field derived from the cross of R32 with a CMS line

Line number	Fertile	Partially fertile	Sterile	d.f.	X^2 1:1:6	Probability
R32-3	2	3	12	2	0.41	0.9-0.75
6	1	4	40	1	5.44	0.1-0.05
8	1	4	31	2	3.37	0.25-0.1
9	0	1	7	2	1.13	0.75-0.5
Summed data	4	12	90	2	7.96	0.025-0.01
		Homogeneity		6	2.34	0.9-0.75
R32-2	14	2	24	2	19.20	0.005

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POLLEN DEVELOPMENT IN MALE-FERTILE AND CYTOPLASMIC MALE-STERILE RYE

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POLLEN DEVELOPMENT IN MALE-FERTILE AND CYTOPLASMIC MALE-STERILE RYE

Abstract

Pollen development in a male-fertile and a cytoplasmic male-sterile line of rye (Secale cereale L.) was investigated using histological techniques. In the male-fertile line a high degree of organization was evident within the locule, and polarity within the microspore was also apparent. In the male-sterile line development appeared to proceed normally until the tetrad stage. Just after tetrad break-up the tapetum became vacuolate and invaded the locule. Two days later the organization within the locule had broken down completely. Microspores and tapetum had become an unorganized mass within the locule. By 10 days after tetrads, the middle layer had also broken down. At dehiscence the contents of the locule remained as a compressed layer over the endothecium of the anther.

Introduction

Laser and Lersten (1972) reported that cytoplasmic male-sterility (CMS) had been documented in approximately 140 species of 47 genera in 20 families of angiosperms. In 38 of these species, the anatomy and/or cytology of microsporogenesis in the CMS form had been studied. The pattern and timing of pollen abortion varied from species to species.

Cytoplasmic male-sterility in rye (Secale cereale L.) has been reported by Russian workers (Zdril'ko, 1969; Kobyljanskij, 1969) and by Geiger and Schnell (1970) and Lapinski (1972). A number of workers have reported that meiosis in CMS forms of rye is normal and that pollen abortion occurs during early pollen development (Belousov and Klyuchko, 1970; Orel, 1972; Gulyaeva, 1972; Kobyljanskij and Katerova, 1972; Zdril'ko and Adamchuk, 1975). Garlicka and Madej (1975) confirmed these earlier findings and were able to detect disturbances in the normal process of tapetal breakdown during pollen development. Degeneration of the tapetum was delayed and contact between the developing pollen grains and the tapetum was lost. However, these authors used Carnoy's fixative which Laser and Lersten (1972) noted probably would induce artifacts. Secondly, they made only a limited number of sections at various stages during pollen development; consequently, direct comparison between fertile and sterile anthers at similar stages of development was not possible. The present study compares pollen development between a male-sterile and a CMS form of rye in an attempt to obtain a better understanding of the process of pollen abortion in this material.

Materials and Methods

Plants of a CMS inbred line of spring rye and its inbred male-fertile maintainer line developed by Scoles and Evans (1979) were grown in a controlled environment cabinet under a 16 hr photoperiod (light intensity 250 microeinsteins m^{-2} sec^{-1}) with light/dark temperatures of 20°C and 15°C respectively. Anthers were obtained as outlined below at the tetrad stage and at 2-day intervals after

tetrad formation. A small "window" was cut in the leaf sheath surrounding a developing inflorescence judged to contain anthers undergoing meiosis. An anther was removed from each primary and secondary floret of a number of spikelets on one side of the inflorescence. After sampling, the leaf sheath was carefully replaced and the incisions were sealed with small pieces of transparent tape to prevent desiccation of the inflorescence. The excised anthers were fixed in Farmer's fluid (Jensen, 1962) and those containing pollen mother cells (p.m.c.) at the tetrad stage of meiosis were identified using aceto-carmine anther squashes. The position of anthers at tetrad stage but on the opposite side of the spike could then be inferred, and these were collected at intervals after tetrad stage. Using this technique, anthers were obtained from both male-sterile and male-fertile forms at tetrad stage, and at 2-day intervals after tetrads through to anthesis (approximately 15 days).

Anthers were fixed under vacuum for 5-24 hr (depending on size) in cold 5% glutaraldehyde buffered with 0.25 M phosphate buffer. After washing in buffer, the anthers were post-fixed in similarly buffered 2% osmium tetroxide for 1-2 hr, then washed and dehydrated in a graded alcohol series and embedded in Spurr's resin (Spurr, 1969). Sections were cut 2 μm in thickness with a Porter-Blum JB4 microtome and stained with 0.1% aqueous toludine blue O. Photographs were taken using a Zeiss Photomicroscope with Kodak HCCF film.

Observations

Figures 1 and 2 illustrate changes occurring in anthers of the male-fertile line. After meiosis, the anther wall consisted of four

layers: epidermis, endothecium, middle-layer and tapetum (Figs. 1a, 1b). Partition walls of the tetrad were formed perpendicular to the tapetum and each tetrad was enclosed in callose. The tetrads were closely packed around the inner face of the binucleate tapetum and each possessed a prominent callose tip which protruded into the centre of the locule.

Two days later (Fig. 1c) the tapetum had decreased in width. The microspores had developed a three-layered wall with a germination pore which was always located on that half of the microspore closest to the tapetum (Fig. 1d). Microspores were in close contact with the tapetum to the extent that the tapetal layer was contiguous with the microspore around its proximal end. Under high magnification, the surface of the tapetum could be seen to be covered in Ubisch bodies (Fig. 1d). Although the microspores had undergone considerable enlargement since their release from tetrads, the diameter of the anther had only increased slightly. As a result the microspores were very crowded and abutted against each other.

Over the next 4 days, the anther increased in diameter, allowing each microspore to occupy more space (Fig. 1e). The single microspore nucleus was usually located at the distal end of the microspore (Fig. 1f). Microspores still remained in close contact with the tapetum which had degenerated further, and Ubisch bodies became more prominent on the surface of the tapetum (Fig. 1f). Eight days after tetrads, the microspores had undergone first pollen grain mitosis (p.g.m.) and a nucleus was located at each end of the microspore (Fig. 2a). The anther had continued to increase in diameter, the

endothecium had developed wall thickenings along inner and radial walls (Fig. 2a), and the tapetum had degenerated to a thin layer no more than 10 μm thick. In some places the microspore was almost in contact with the endothecium, however, nuclei were still present in the tapetum (Fig. 2a). At 12 days after tetrads, the generative nucleus (in the distal end of the microspore) had become enlarged and took on the appearance of a prophase nucleus (Figs. 2b, 2c). The synthesis of starch granules had begun (Fig. 2b). The generative cell and nucleus later moved towards the vegetative nucleus in the proximal end of the microspore (Fig. 2d). As a result of this movement, cytoplasm became localized in the proximal end of the microspore (Fig. 2d). Fourteen days after tetrads (just prior to anthesis), most microspores were non-vacuolate and the cytoplasm was filled with starch grains (Fig. 2e) which made observation of the vegetative and two generative nuclei difficult; however, under high-power some nuclei were always present at the distal end of the microspore (Fig. 2f). Traces of tapetal tissue were still present.

Anthers of the male-sterile line were not obtained at the tetrad stage; however, anthers were obtained at diakinesis (Fig. 3a). There was no evidence of disturbance in either the p.m.c. or the tapetal cells at diakinesis. The p.m.c. were enclosed in callose and each bore a tip of callose which protruded into the locule (Fig. 3a). The tapetum appeared to be comparable to the tapetum of the fertile anther at tetrad stage. However, just after tetrad break-up the cytoplasm of tapetal cells became vacuolate and cell walls between some adjacent cells broke down, resulting in a coenocytic structure which invaded

the locule (Fig. 3b). This structure was contained within a membrane which appeared to be continuous with the inner tapetal membrane. The contained nuclei were of normal appearance. Although all traces of callose around the microspores had gone, the microspores had still retained their shape from tetrads. Two days after tetrads (Fig. 3c) the internal organization of the locule had broken down. The epidermis, endothecium and middle-layer remained intact but the tapetum and microspores had become an unorganized mass. Nuclei and microspore shells were still evident in the mass. Over the next 4 days, the mass became compressed and was distributed around the locule as a thin layer on the surface of the middle-layer (Figs. 3d, 3e). By 8 days after tetrads, the middle-layer had also broken down (Fig. 3f). The anther remained in this condition throughout the remaining period of microspore development. Although intact, the walls of the endothecium did not develop thickenings and the locule did not split open at the time of anthesis. At this time the sterile anthers were shorter than fertile anthers, white in colour and were retained within the floret. Fertile anthers, on the other hand, were pushed out of the floret by filament extension just prior to dehiscence.

Discussion

The development of pollen in male-fertile rye has not previously been studied in such detail in sectioned material. Thus, before discussing the features of pollen development in the male-sterile line, we would like to relate events in the male-fertile line to previous reports concerning pollen development in rye and in other species.

Pollen Development in the Male-Fertile

Bennett and Smith (1972) investigated pollen maturation in rye as part of a study on the effects of polyploidy on meiotic duration and pollen development. They estimated the period from second telophase to first p.g.m. to be 6 days, from first to second p.g.m. to be 4.5 days and from second p.g.m. to dehiscence to be 5.5 days. In the present study first p.g.m. occurred between 6 and 8 days after tetrads, second p.g.m. occurred between 12 and 14 days after tetrads and dehiscence occurred about 15 days after tetrads. Although the period from tetrads to dehiscence was similar in the two studies, the intervals between meiosis, first p.g.m. and second p.g.m. were longer in the present study and the interval between second p.g.m. and dehiscence was shorter. These differences are presumably due to environmental effects (Bennett and Smith grew their material at 20°C in continuous light) and genotypic effects.

Our observations indicated that a high degree of organization existed within the locule. From meiosis to dehiscence the developing microspores remained in close contact with the tapetum. Similar contact was observed to exist in the anther of sorghum (Christensen and Horner, 1974). The orbicules of the tapetum and the sporopollenin of the microspore were implicated in this attachment. Christensen and Horner (l.c.) also reviewed the occurrence of polarity within the microspores of members of the Gramineae. Polarity in both p.m.c. and microspore was evident in the present study.

The first evidence of polarity within the p.m.c. was the consistent formation of the partition walls of the tetrad perpendicular to the tapetum. As a result, each microspore of a tetrad retained contact

with the tapetum. Similar observations have been made in other members of the Gramineae (see Christensen and Horner, 1974). Polarity within the p.m.c. was carried over into the microspores. This was first observed 2 days after tetrads when it was noted that the germination pore of the microspore was always located on that half of the microspore adjacent to the tapetum. Wodehouse (1935) described the pore of a rye pollen grain as occurring "on one side near the end" in agreement with the present observations. Orientation of the pore towards the tapetum has been observed in other members of the Gramineae (see Christensen and Horner, 1974). In sorghum, the pore was always located immediately adjacent to the tapetum (Christensen and Horner, 1974), however, in the present study it was never observed in direct contact with the tapetum. Banerjee and Barghorn (1971) are of the opinion that orientation of the pore towards the tapetum is a result of reorientation of the microspores within the locule.

Polarity within the microspore has been observed by both Bennett et al. (1973) and Christensen and Horner (1974). In sorghum microspores vacuolization consistently displaced the single nucleus to the distal end of the microspore, opposite the pore, where first p.g.m. occurred (Christensen and Horner, 1974). In wheat, however, Bennett et al. (1973) observed that the single microspore nucleus lay under the pore. In the present study, 6 days after tetrads and just prior to first p.g.m. the microspore nucleus was observed more frequently at the distal end of the microspore, opposite the pore, as in sorghum.

In both sorghum and wheat, nuclear movement after first p.g.m. resulted in distribution of one nucleus to each end of the microspore (Christensen and Horner, 1974; Bennett et al., 1973 respectively).

In these species the vegetative nucleus was located at the proximal end of the microspore (the end possessing the germination pore) while the generative cell and nucleus were at the distal end. This separation of the nuclei was also observed in the present study. Prior to second p.g.m., Christensen and Horner (1974) observed movement of the generative cell towards the vegetative nucleus. Similar movement was also observed in the present study.

It seems apparent that the developing microspore of members of the Gramineae has a definite polarity which determines pore formation and both nuclear and cytoplasmic movement. Wodehouse (1935) noted that pore formation in pollen was not random and related the arrangement of pores to the geometry of contact between microspores in a tetrad, which in turn would be dependent on the planes of division during meiosis. Dover (1972) observed pore formation in wheat microspores to occur adjacent to spindle poles at telophase II and postulated that spindle formation and pore formation were predetermined by a self-replicating cytoplasmic factor(s). Christensen and Horner (1974) discounted this hypothesis and suggested that events during the pre-meiotic interphase were responsible for inducing polarity. More specifically, they postulated that as callose enveloped the pre-meiotic p.m.c., regions of the p.m.c. adjacent to the tapetal surface were the last to be enclosed. Thus, these regions remained receptive to outside influences longer than other areas of the p.m.c. and it was suggested that this process established the polarity observed in the p.m.c. and its microspores.

Pollen Development in the Male-Sterile

Anthers of the CMS line at early meiosis appeared to be free of abnormalities in either the p.m.c. or the tapetum. This supports earlier observations that meiosis in CMS rye lines was normal (Belousov and Klyuchko, 1970; Orel, 1972; Gulyaeva, 1972; Kobyljanskij and Katerova, 1973; Zdril'ko and Adamchuk, 1975; Garlicka and Madej, 1975). A normal meiosis in CMS forms has been found to be common among the Gramineae. Laser and Lersten (1972) reviewed the anatomy and cytology of CMS forms in 24 members of the Gramineae and in only one case was meiosis disturbed. Meiotic disturbances were more frequent in dicotyledonous species.

In the present study, the first evidence of abnormality in the anthers of the CMS line occurred just after tetrad break-up when the tapetal layer became vacuolate and coenocytic and invaded the locule. Vacuolation and enlargement of the tapetum has frequently been reported to occur in CMS lines and generally is of two types. In one type, the cellular nature of the tapetum remains intact and vacuolation in each cell causes hypertrophy, sometimes to such an extent that the locule is occluded and the developing microspores crushed. This has been observed in CMS beets (Artschwager, 1974), corn (Chang, 1954), onion and radish (Nishi and Hiraoka, 1958), wheat (Chauhan and Singh, 1966), cucumber (Chauhan and Singh, 1968), sorghum (Overman and Warmke, 1972), pepper (Horner and Rogers, 1974) and sunflower (Horner, 1977). In the second type of behavior the cellular nature of the tapetum breaks down and the cell contents fuse to form a coenocytic periplasmodium which invades the anther locule, such as in the present study. This structure is always contained within a membrane continuous with the inner wall

of the tapetal layer, thus distinguishing it from the periplasmodial invasive tapetum that occurs in some plant species during normal sporogenesis (Maheshwari, 1950). Overman and Warmke (1972) observed such a structure in CMS sorghum and termed it an intratapetal syncytium. Intratapetal syncytia have also been observed in CMS beets (Artschwager, 1974), carrots (Zenkteler, 1962), flax (Dubey and Singh, 1965) and wheat (Chauhan and Singh, 1966; Rai and Stoskopf, 1974).

Garlicka and Madej (1975) concluded that delayed degeneration of the tapetum was the cause of sterility in a CMS line carrying Pampa cytoplasm (the same cytoplasm as incorporated into the line used in the present study) and also in another CMS line. The tapeta of both CMS lines were still cellular when only vestiges of the tapetum remained in a male-fertile line. In the line carrying Pampa cytoplasm contact with the tapetum was lost as early as tetrads. In the other line, disturbances were first detected after tetrad break-up, when microspores lost contact with the tapetum. Once contact was lost, degeneration of the microspores occurred. The pattern of breakdown observed in the present study does not seem to be the same as that observed by Garlicka and Madej. They did not detect the formation of intrapetal syncytia and they show quite clearly an anther with highly degenerated microspores but intact tapetum. These differences are not easily explained, although they may be due to the different genotypes of the material used and to different environmental conditions during development.

Cebrat and Zadecka (1978) have carried out a study of pollen development in three genetic male-sterile lines of rye. Unlike CMS rye, they observed disturbances in anther morphology and disturbances

during meiosis in all three lines. In two lines, changes in the tissue surrounding the vascular bundle of the anther (in one line development of an endoderm, in the other suberization of walls) were thought to have reduced nutrient supply to the pollen chambers. In the third line the tapetal layer remained uninucleate and became highly vacuolate, taking on the appearance of the hypertrophied tapetal layer seen in some CMS lines. In one line intratapetal syncytia identical to those observed in the present study were sometimes detected.

The importance of the production and breakdown of callose to the development of microspores has been emphasized by Heslop-Harrison (1971). Mistiming of callose dissolution has been suggested as a cause of CMS in Petunia (Izhar and Frankel, 1971). In CMS sorghum Warmke and Overman (1972) also found aberrant callose behavior which they ascribed to premature callose activity. In the present study there was no evidence of either premature or delayed callose dissolution. In both species known to exhibit premature dissolution this has been detected during meiosis (Frankel et al., 1969; Warmke and Overman, 1972) whereas in the present study callose appeared to be normal during meiosis in the CMS line. By the time of tetrad breakup all traces of callose had disappeared, thus there was no evidence of late callose dissolution in this material.

In the present study, as in many others (Laser and Lersten, 1972; Horner and Rogers, 1974; Horner, 1977) the sequence of events in the anther is highly suggestive of failure of the tapetum as a causative agent in pollen abortion. It is now well established that the tapetum serves a major role during microsporogenesis although the full extent

of this involvement either through nutrition of the microspores or production of microspore wall components or both still remains unclear. However, it is also possible that these changes in the tapetum are initiated by earlier undetected changes involved in the breakdown of the microspores. A solution to this problem seems to rely upon a better understanding of the role of the tapetum and its interaction with sporogenous tissue, and ultimately upon a full understanding of how an interaction between nucleus and cytoplasm can affect abortion of the male gametophyte while other cells and tissues of a plant develop normally.

Figure 1. Sections of male-fertile anthers; a) at tetrad stage x600; b) as in a) but x1512 (a=epidermis, b=endothecium, c=middle layer, d=binucleate tapetum, e=sporocytes still in tetrads, f=callose); c) at tetrads + 2 days x600; d) as in c) but x2400 (u=developing ubisch bodies over tapetum, p=germination pore); e) at tetrads + 6 days x600; f) as in e) but x2400 (u=ubisch bodies).

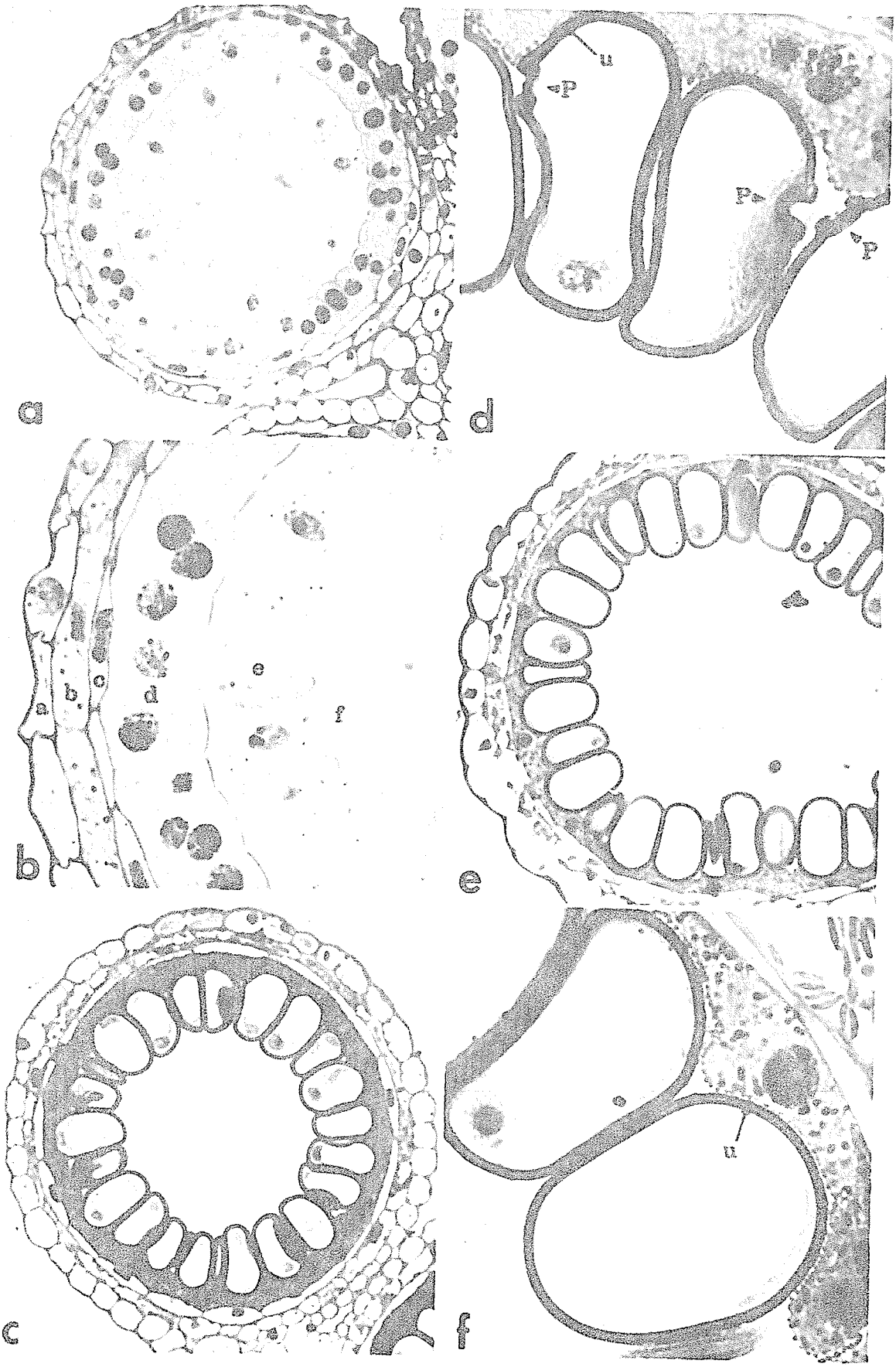
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Figure 2. Sections of male-fertile anthers; a) at tetrads + 8 days x1512 (u=ubisch bodies, th=thickenings in radial walls of endothecium); b), c), and d) at tetrads + 12 days x1512 (b) and c) prior to movement of generative nucleus towards vegetative nucleus, d) after movement of generative nucleus towards vegetative nucleus); e) at tetrads + 14 days x600; f) as in e) but x1512 (n=nuclei in distal end of microspore.

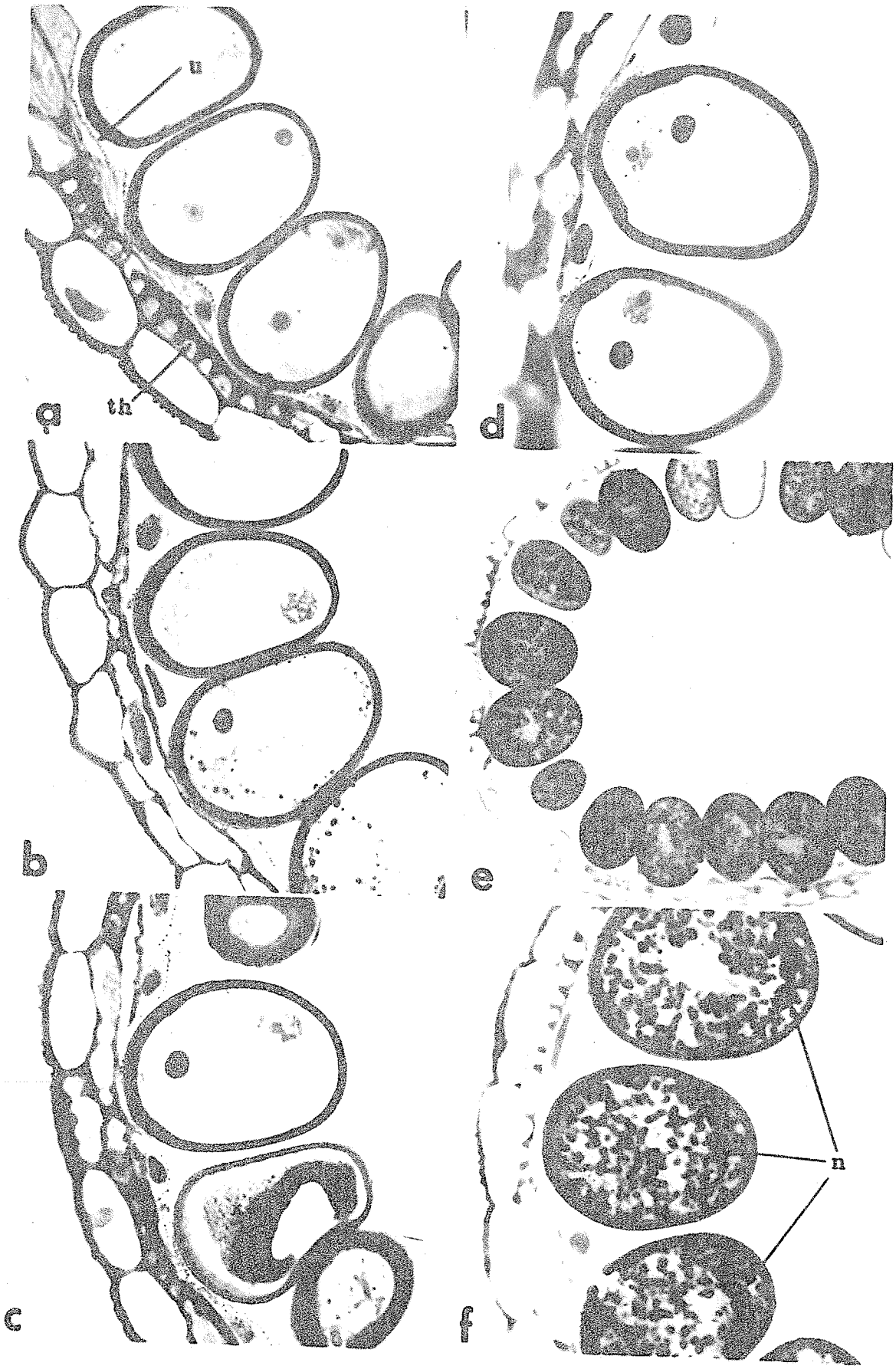
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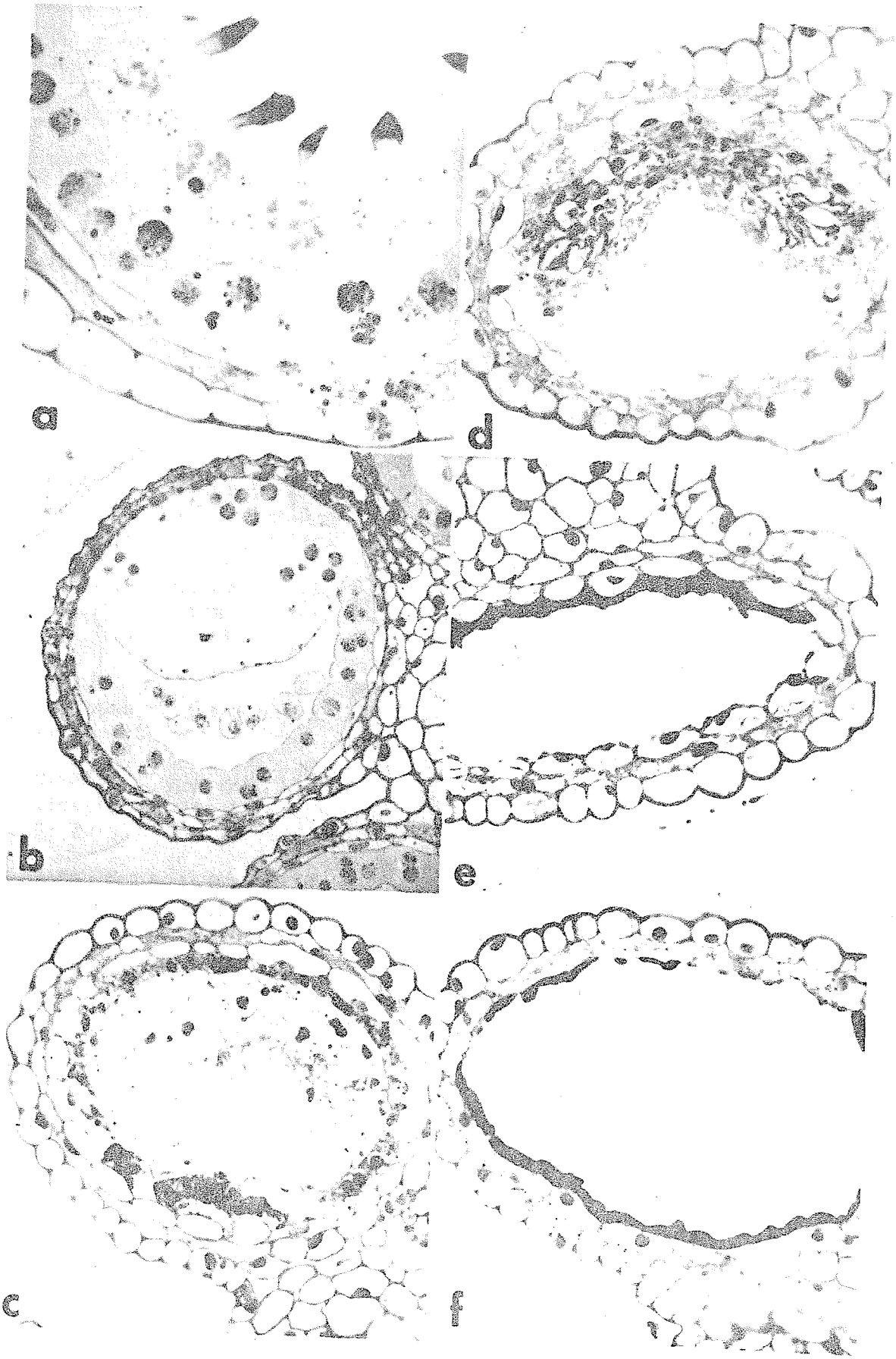
Figure 3. Sections of male-sterile anthers; a) at diakinesis x1512; b) after tetrad break-up x600; c) at tetrads + 2 days x600; d) at tetrads + 4 days x600; e) at tetrads + 6 days x600; f) at tetrads + 8 days x600.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GENERAL DISCUSSION

Cytoplasmic male-sterility induced by Pampa cytoplasm was successfully transferred from winter to spring rye. From the small sample of inbred lines selected for study (six), lines either homozygous for maintenance of the sterility or homozygous for restoration of fertility were obtained after three or more generations of inbreeding. All except one of the inbred lines utilized were heterozygous with respect to restorer genes. The frequency of restoration in F_1 progenies from crosses between these lines and the cytoplasmic male-sterile varied from 20-69%. In the open-pollinated cultivar Gazelle a restorer gene frequency of 66% was observed. These values are similar to those reported by Geiger (1972). He crossed 10 open-pollinated cultivars with lines carrying the Pampa cytoplasm; the frequency of fertility restoration genes varied from 20-50%. Thus, it should theoretically be possible, with inbreeding and selection, to obtain homozygous maintainer and restorer lines from any open-pollinated population. However, this process was unsuccessful when undertaken in the present study because of the very low self-fertility of open-pollinated material and the high mortality of any progeny obtained upon selfing. Success in transferring the sterility and isolating new restorer lines is more likely to be achieved through selection within inbred material. It is apparent from the present study that despite

at least seven generations of inbreeding heterozygosity with respect to restorer genes still remained.

The present study indicated that at least three complementary dominant genes were operative in restoring fertility. For full fertility the dominant allele of all three genes needed to be present except in the most favourable environment, when two dominant alleles sufficed. However, the genes were not of equal expressivity; only certain genotypes possessing dominant alleles at two of the three loci were fully fertile in the favourable environment. Geiger (personal communication) has also found up to three restorer genes although in some combinations of sterile and restorer a single gene appears to restore full fertility. His results also indicate that the genetic background of a line can modify the action of restorer genes. Fertility restoration to other cytoplasmic male-sterility systems developed in rye, that utilize alternate sterilizing cytoplasms, has in some cases been found to have a simple genetic basis (Kobyljanskij, 1969) but in others it appears to be more complex (Zdril'ko and Adanchuk, 1975). Thus, the genetic basis of fertility restoration to cytoplasmic male-sterile rye appears to be comparable to that of corn in which either one or two major genes restore fertility depending upon the source of sterilizing cytoplasm (Duvick, 1965). The system in rye appears to be simpler than fertility restoration in wheat (see Literature Review). This may be due in part to the polyploid nature of wheat.

The Pampa cytoplasmic male-sterility was stable over environments; anther development was always poor and pollen production within an anther was always zero. Fertility restoration, however, was sensitive

to environment. Restoration was best under temperature regimes of 20/15 or 25/20°C rather than at 15/10°C. Geiger (personal communication), however, suggested that stress-free growth (low temperature, non-limiting moisture, high light and high fertility) resulted in maximum restoration. This seems to be supported by the fact that in the genetic study certain genotypes which were either fertile or partially fertile in the growth room were respectively partially fertile or sterile in the greenhouse or field. Presumably, in the latter environments plants would be subject to more stress than in the growth room. Best restoration at low temperatures has also been reported in wheat (Johnson and Patterson, 1973), corn (Duvick, 1965) and Petunia (van Marrewijk, 1969). The apparent contradiction between the results of the environment study and the generally recognized effects of environment on fertility restoration can best be explained by assuming that neither of the three temperature regimes used were extreme enough to significantly affect the fertility of the restored lines. The sterility observed in this experiment must then be genetic in origin, presumably due to heterozygosity in the restorer line used. The cause of this heterozygosity is unknown.

This study indicated that sterility was accompanied by tapetal disturbance. After tetrad break-up the tapetum invaded the anther locule and within 2 days the organization of the locule had been lost. Prior to this development within the anther appeared to be normal. The timing of events could suggest that the tapetal disturbance is the cause of sterility as microspores were developing normally when it occurred. This conclusion was also reached by Horner (1977)

studying cytoplasmic male-sterile sunflower. He reviewed studies of cytoplasmic male-sterility in another 12 species having this pattern of breakdown. Warmke and Lee (1977) reported that the tapetal mitochondria of cytoplasmic male-sterile maize (T cytoplasm) degenerated shortly after meiosis while microspore development was normal. Similar observations have since been made in sunflower (Horner, 1977) and beets (Nakashima, 1978) suggesting that the initial cause of tapetal disturbance is the malfunctioning of tapetal mitochondria. Recently differences in the mitochondrial DNA of both S- and T-type cytoplasmic male-sterile maize have been reported (Levings and Pring, 1976; Pring et al., 1977) and presumably these differences are the cause of sterility. Why these changes are only expressed in the tapetum at a certain stage is not known. Flavell (1974) proposed a model for cytoplasmic male-sterility in which it was suggested that "a substance(s) present only in the anther interact with cytoplasmic organelles (either mitochondria or chloroplasts) having an altered structure to prevent normal anther development", however, there is as yet no evidence as to the nature of this substance(s).

This cytoplasmic male-sterility has obvious potential as a tool in hybrid rye production. Although it has been well documented that substantial heterosis is exhibited by hybrids of inbred lines, any yield advantages of hybrids over current Canadian out-pollinated cultivars has been found to be marginal (Scoles and Evans, 1978; McLeod, 1979). Thus, it seems questionable whether the work and money required to develop inbred lines and to test hybrids could be warranted. Furthermore, due to the small acreage of rye grown in Canada rye breeding has previously been given a low priority in cereal

breeding programs. It seems reasonable to suggest that significant yield advances could be made through a more intensive program aimed at producing new out-pollinated cultivars without resorting to hybrid cultivars. In view of the fact that the use of a single source of cytoplasmic male-sterility in corn was the primary cause of epiphytotics of southern corn leaf blight and yellow leaf blight (Helminthosporium maydis and Phyllosticta maydis respectively) (Scheifle et al., 1969), it would also seem unwise to promote cultivation of hybrid rye through the use of cytoplasmic male-sterility until a number of other sources of sterilizing cytoplasm are available.

It is conceivable that the cytoplasmic male-sterility might be of use in a rye breeding program aimed at producing open-pollinated cultivars. Conversion of a broad-based tester line having high general combining ability (G.C.A.) to the cytoplasmic male-sterile condition would allow topcrosses between that tester and a large number of lines to be made simply by growing these two lines in isolation. Lines established as having good G.C.A. using this system could be bulked to form a synthetic cultivar.

SUGGESTIONS FOR FURTHER STUDY

Further study in each of the three areas investigated in this thesis is recommended:

Genetics - analysis of F_2 and backcross progeny produced with other restorer lines developed from new sources;

- analysis of progeny from selfed partially fertile plants.

Stability - analysis of the fertility of sterile/restorer hybrids over a greater temperature range, at different levels of illumination and at different levels of humidity;

- analysis of the period during growth when environment is most critical.

Histology - an electron-microscope study of tapetal ultrastructure during meiosis and early microspore development in cytoplasmic male sterile lines.

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