STUDIES WITH CYTOPLASMIC MALE-STERILE RYE

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Graham John Scoles

In Partial Fulfillment of the

Requirements for the Degree

of

Doctor of Philosophy

Department of Plant Science

October 1979

STUDIES WITH CYTOPLASMIC MALE-STERILE RYE

BY

GRAHAM JOHN SCOLES

A dissertation submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

DOCTOR OF PHILOSOPHY
© 1979

ACKNOWLEDGMENTS

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.

TABLE OF CONTENTS

LIST OF TABLES

<u>The Genetics of Fertility Restoration in
Cytoplasmic Male-Sterile Rye</u>

LIST OF FIGURES

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

An attempt was made to transfer cytoplasmic male-sterility from
winter rye (<u>Secale cereale</u> 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 homo-
zygous for maintenance of sterility or for restoration of fertility
were obtained from some inbred lines. During this process environ-
mental 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 incor-

porating 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 (1.c.) reported that 15 sources of cyto-plasmic 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 back-cross 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 demon-strated 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 develop-
ment 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 chromósomal 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