To the Graduate Council:

I am submitting herewith a thesis written by Gary E. Day entitled "Inheritance of Sterility in Mutants of Soybeans." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

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INHERITANCE OF STERILITY IN MUTANTS OF SOYBEANS

A Thesis

Presented for the

Master of Science

Degree

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Gary E. Day

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ABSTRACT

In the summer of 1978 crosses were made between maintainer lines of ms_1 and ms_2 and a soybean mutant of D68-127 segregating for sterility. D68-127 originated from the cross Dyer/Bragg. Sterile mutants of D68-127 resulted from treatment of seed with the mutagenic agents diethyl sulfate and fission neutrons.

F_1 plants were grown in the greenhouse during the winter of 1978-79 and F_2 populations were grown in the field during the summer of 1979. Near maturity, all plants were classified on the basis of phenotype as being fertile or sterile.

The objective of this study was to test the hypothesis that the sterility mutant of D68-127 is a male-sterile mutant conditioned by a single gene, either ms_1 or ms_2. Based on the results obtained it appears that the gene involved in the sterility of D68-127 is a single recessive gene and that it is male-sterile. The F_2 segregation data best support ms_1 as being the gene involved in the sterility of D68-127. However, the possibility of ms_2 or other ms genes could not be ruled out. Sterile plants, or those remaining green at maturity, of ms_1, ms_2 and D68-127 mutant produced variable amounts of viable pollen which resulted in seed production in many instances indicating that none of the genes provided complete male-sterility.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Sterility has been observed in almost all diploid species of plants. The occurrence of such plants is rare and often involves both male and female-sterility. Various degrees of sterility have been found in soybeans ranging from completely sterile, to partial male-sterile and partial female-fertile, to completely male-sterile and completely female-fertile. Owen (16), in 1928, reported finding completely sterile soybeans in which both ovule and pollen grains were nonfunctional. Woodworth (29), in 1933, and Morse and Cartter (15) in 1937, referred to this sterile character as st. In 1964, Hadley and Starnes (12) reported separate sterile characters in two lines, T241 and T242. The gene symbols st_2 and st_3 were assigned to T241 and T242, respectively. Sterility was due to asynapsis, the lack of chromosome pairing in the first meiotic prophase. Sterility was associated with the homozygous condition of a recessive gene.

Allelism tests by Palmer (18) of the sterility mutant in the cultivar Hark determined that the gene causing sterility was at a different locus than st_2 and st_3. This gene was assigned the symbol st_4 and is maintained in the Soybean Genetic Type Collection as T258H. Cytological studies by Palmer (18) showed that homologous chromosomes were paired initially and that sterility resulted from the failure of homologous chromosomes to remain paired throughout meiosis. Thus,

st_4 is classified as a desynaptic mutant as opposed to the asynaptic mutants st_2 and st_3.

In 1979, Palmer (19) reported on a sterile appearing plant found at Ames, Iowa, which resembled the st series. Cytological studies and allelism tests provided evidence that this was either an asynaptic or desynaptic mutant and that the gene responsible was different from st_2, st_3 and st_4. This new gene was assigned the symbol st_5 and is maintained in the Soybean Genetic Type Collection as T272H.

Brigham (4) has recently reported finding a sterile mutant in the progeny of a Forrest/Kent cross. This mutant resembles the st series. It has been tentatively named "TAES Lubbock Sterile" until allelism tests can be completed.

The first report of a partially-sterile plant was provided by Stewart and Wentz (28) in 1926. These partially-sterile plants were characterized by the lack of pubesence, small crinkled leaves, dwarfed condition and a very small number of pods per plant. The gene responsible for this sterility was assigned the symbol p_2.

Caviness et al. (8) in 1970 observed a partially male-sterile strain of soybeans. These plants retained their leaves at maturity and resembled virus infected plants. The authors used approach grafts of partially male-sterile and normal plants to verify that infective agents were not involved. Pollen of the partially male-sterile plants was small and shrunken. Pollen stained in a solution of acetocarmine and glycerine indicated that only 5% to 10% of the pollen produced was viable. Only crosses using the partially male-sterile plants as the

female were successful. Natural cross-pollination was 45 times greater in the partially male-sterile strain. Using pubescence color as a marker, it was found that 0.26% natural cross-pollination occurred in the fertile strain compared to 11.21% for the male-sterile strain. Caviness and Fagala (9) found that temperature affected the degree of sterility in the Arkansas partially male-sterile strain. This strain set no pods when the day temperature was 35C and night temperature was 21 or 27C. However, at day temperatures of 21 or 29C and night temperatures of 16 or 21C, plants were partially fertile. The largest number of pods was produced when the day temperature was 29C followed by night temperature of 21C.

Stelly and Palmer (26) recently reported on a partially male-sterile mutant controlled by a single recessive allele. Expression of this character is highly variable depending on environment and genotype. Seed set on genetically sterile plants varies from a few to normal amounts. This gene has a pleiotrophic effect on flower morphology such that standard petals do not bend back but enclose the wing and keel petals. This mutant is maintained in the Soybean Genetic Type Collection as T271H.

The sterile mutant of most practical use is the complete male-sterile female-fertile type. The first sterility of this type was reported by Brim and Young (6) in 1971. At present four different genes are known that when present in the homozygous recessive condition result in completely male-sterile, female-fertile plants. These genes are designated ms_1, ms_2, ms_3 and ms_4. The sterile character identified

by Brim and Young (6) was essentially completely male-sterile and conditioned by a single recessive gene. They assigned it the gene symbol ms_1. Sterility was determined to be the result of nonfunctioning pollen. The male-sterile plants could not be distinguished from fertile siblings until pod set, at which time flower drop and failure of pod development was observed. With the onset of maturity, striking differences could be observed between fertile and sterile plants. Male-sterile plants failed to mature normally and were characterized by low seed set, fleshy pods, and dark green leathery leaves which were not shed until frost. Often there was a proliferation of vestigial pods at flowering nodes. Anther and pollen development appeared normal. Pollen tube development did not occur in male-sterile plants. Pollen of male-sterile plants followed a characteristic clumping pattern in solution. Dehiscence of anthers on male-sterile plants did not occur as easily as on fertile plants. They observed that male-sterile plants grown in the greenhouse were barren, while male-sterile plants in the field usually produced a few fleshy pods. It was determined by means of a natural crossing block that 99% of the seed that occurred on male-sterile plants were the result of cross-pollination. The lack of seed on greenhouse grown male-sterile plants was attributed to a lack of pollen vectors; however, the possibility of partial female-sterility could not be discounted.

Palmer and Albertsen (22) found that male-sterile (ms_1) plants produce twice as many pollen mother cells as do fertile siblings. Pollen mother cells of male-sterile plants are approximately the

same size as those of fertile plants. Anthers of both male-sterile and fertile plants have four locules, however, anthers of male-sterile plants are larger due to the increased number of pollen mother cells. Because ms_1 male-sterile plants do not undergo cytokinesis they produce only as many pollen grains as they have pollen mother cells, whereas, fertile plants produce four pollen grains for each pollen mother cell. The ratio of pollen grains per locule of male-sterile plants versus fertile plants was 1:2 instead of the expected 1:4.

Boerma and Cooper (3) observed male-sterility in the F_3 generation of the cross L67-533/SRF300 and designated it as the Urbana male-sterile. Reciprocal crosses made between known heterozygotes of ms_1 and Urbana male-sterile indicated that the Urbana male-sterile is controlled by a single recessive gene which appears to be at the same locus as the ms_1 gene. It was observed that Urbana male-sterile plants characteristically had a much higher seed set and frequency of 2- and 3-seeded pods than did ms_1 sterile plants. Results from two environments indicated that Urbana male-sterile and ms_1 are phenotypically distinct based upon the frequency of 3-seeded pods. Since they are phenotypically different it may be that the Urbana male-sterile is a different allele than ms_1 or that it has closely linked modifier gene(s) which affect female-fertility. The Urbana male-sterile is maintained in the Soybean Genetic Type Collection as T266H.

Albertson and Palmer (1), working with the Urbana male-sterile, found that environmental influences were significant in causing variation in the number of pollen grains per flower of fertile and sterile plants.

They concluded that a single characteristic ratio of normal pollen grains to coenocytic pollen grains cannot be established for the ms_1 gene in the Urbana male-sterile.

Palmer (17), in 1974, observed that pollen from an off-type plant in T258 (st_4) was large and dark staining in contrast to the small, collapsed, non-starch-filled pollen normally produced by st_4 sterile plants. This line was designated the Ames male-sterile.

Palmer et al. (23) made allelism tests among ms_1, Ames, Urbana, and Tonica male-steriles. Tonica male-steriles were observed in a commercial field of Harosoy in 1955. In all cases, known heterozygous males crossed onto homozygous recessive females resulted in a 1 fertile: 1 sterile F_1 ratio, with a 3 fertile: 1 sterile F_2 ratio from the fertile F_1 plants. In all cases the pollen was characteristic of that produced by ms_1. These results indicate that Ames, Urbana, and Tonica male-steriles are all at the same locus as the ms_1 mutant, however, it is not known if they are all the same allele or part of a multiple allelic series.

Bernard and Cremeens (2) observed a few poorly podded plants in the F_3 generation of the cross SL11/L66-177. A high number of seeds per pod was observed on these plants. Anthers of these plants were very small and no pollen was produced. Sterile plants averaged nearly 87% fewer seed per plant as fertile siblings. Pollinations made onto fertile plants of this strain averaged 51% seed set, whereas, polliantions made onto sterile plants averaged 42%. Thus, female-fertility appears to be near normal in this strain. In a similar

situation pollinations made onto ms_1 ms_1 plants averaged only 16% seed set. Allelism tests were conducted with this sterile and ms_1 using known heterozygotes as males and male-sterile as females. Fifty percent of the segregating F_2 generation fit a 3 fertile: 1 sterile ratio and 50% fit a 9 fertile: 7 sterile ratio indicating that the genes causing sterility in this strain and ms_1 are at separate loci and are not linked. This form of male-sterility was assigned the gene symbol ms_2 and added to the Genetic Type Collection as T259H. It became known as the Eldorado male-sterile.

Chaudhari and Davis (10) reported finding male-sterile plants in the cultivar Wabash. A few seed were harvested from these plants and advanced through the F_2 generation. Segregation in the F_2 indicated that this sterility was due to a single homozygous recessive gene. The Wabash male-sterile was found to produce no pollen, similar to ms_2. The Wabash male-sterile is maintained as SB-12.

Palmer (20) observed sterile appearing plants in F_3 progeny of the cross Calland/Cutler. Allelism tests with ms_1 and ms_2 resulted in F_2 populations in which half of the families segregated 3 fertile: 1 sterile and half segregated 9 fertile: 7 sterile in each case, indicating that the gene responsible for male-sterility is at a different locus than ms_1 and ms_2. This new gene was given the symbol ms_3 and added to the Soybean Genetic Type Collection as T273H. Male-sterility in ms_3 is the result of a breakdown of microspores as soon as they are released from the tetrad. Female-fertility appears near normal as evidenced from pollinations made on sterile plants.

Palmer (21) observed a sterile appearing plant in a field of Rampage soybeans. This plant produced three seed. Allelism tests with ms_1, ms_2, and ms_3 using sterile plants as females and known heterozygotes as males resulted in all fertile F_1s and F_2 populations in which half of the families segregated 3 fertile: 1 sterile and half segregated 9 fertile: 7 sterile in all cases. These results indicated that the gene responsible for sterility in Rampage soybeans is at a different locus than any of the previously discovered ms mutants. This gene was assigned the symbol ms_4 and added to the Soybean Genetic Type Collection as T274H. Female-fertility of ms_4 appeared to be normal as pollinations on fertile and sterile plants were equally successful.

Various other sterile and/or male sterile types have been reported. Hadley et al. (13) observed segregation for partial sterility in the cultivars Calland, Wayne and Woodworth. Segregation ratios suggested that all three are male-steriles. All three were said to have had empty anthers and set relatively high numbers of one-, two-, three- and four-seeded pods indicating high female fertility.

Stelly et al. (27) found two spontaneous occurring sterile plants which did not have any genetic system for sterility. They suggest that the steriles arose from plants with highly elevated chromosome numbers.

To date, all ms mutants have been found to occur naturally. However, it is known that irradiation treatments can produce sterility in soybeans. Singh et al. (25), irradiated the varieties Clark 63, Lee, Bragg and Semmes in 1970. Two M_3 progenies of Semmes were found

to segregate for sterility. These plants remained green until frost and produced only a few pods. These progenies were designated Semmes M.S.1 and Semmes M.S.2. Studies conducted with Semmes M.S.1, Semmes M.S.2 and ms_1 indicated that all were governed by a single recessive gene. However, pollen studies and the difference in pod set observed among the lines indicated that the gene for sterility was different in all 3 lines. Semmes M.S.1 produced normal sized, nonfunctional pollen, and set 0-5 pods per plant indicating some female-fertility. Semmes M.S.2 produced no pollen but set from 5-85 pods per plant indicating greater female-fertility. The sterile ms_1 plants produced nonfunctional pollen grains that were larger than normal. These plants set from 1-50 pods per plant. Cytological studies by Patil and Singh (24) revealed specific abnormalities in these lines. Normal cytokinesis occurred in Semmes M.S.1, however, the microspores failed to develop normally resulting in nonfunctional pollen grains. Normal cytokinesis also occurred in Semmes M.S.2, but the daughter nuclei immediately degenerated and no microspores were formed. Cytokinesis failed to occur after telophase II in ms_1 resulting in the 4 daughter nuclei being encapsulated within a single large nonfunctional pollen grain.

Further irradiation treatments by Jha and Singh (14) led to the discovery of seven additional lines segregating for sterility. Sterility in these lines was of three types: complete male- and female-sterility, complete male-sterility and partial female-fertility, and partial male-sterility and complete female-fertility. In all cases the inheritance of sterility was due to a single recessive gene. Allelic relationships among the seven lines are not yet known. Sterility in PK-71-39 S-1 and

PK-71-39 S-2 was due to asynapsis. Desynapsis appeared to be the cause of sterility in Ankur S-1. Sterility in Bragg S-1 was due to the failure of bivalents to properly align on the metaphase I plate, resulting in unequal distribution of chromosomes. All four of the above abnormalities resulted in complete male- and female-sterility. Complete male-sterility and partial female-fertility of UPSM-229 MS-1 resulted from improper alignment of bivalents at metaphase I. Complete male-sterility and partial female-fertility of UPSM-299 MS-2 resulted from failure of cytokinesis similar to ms_1. Partial male-sterility and complete female-fertility of UPSL-18 MS-1 was due to chromatids remaining together during anaphase II. Sterility in these seven lines as well as Semmes M.S.1 and Semmes M.S.2, and ms_1 is due to a particular mutation affecting a particular step of sporogenesis.

Several uses may be envisioned for a male-sterile character. The possibility exists for using ms_1 plants to produce aneuploids for cytogenetic research. Brim (5) and Brim and Stuber (7) suggest that the greatest potential of male-sterility in soybeans is for making the large number of crosses needed for a recurrent selection program. The use of male-sterility in such a program reduces the labor of hand pollination thereby providing a means for more efficient development of diverse germplasm pools. Preliminary results indicate a significant increase in yield from the recurrent selection procedure proposed by Brim and Stuber (7).

The objective of this study is to test the hypothesis that the sterility mutant of D68-127 is a male-sterile mutant conditioned by a single gene, either ms_1 or ms_2.

CHAPTER II

MATERIALS AND METHODS

In the spring of 1974 seed of the experimental line D68-127, a selection from the cross Dyer/Bragg, were treated with mutagenic agents at The University of Tennessee's Comparative Animal Research Laboratory, Oak Ridge, Tennessee. Treatments with two mutagenic agents induced sterile plants used in this study. The first treatment consisted of soaking seed of D68-127 in diethyl sulfate (DES) for 90 minutes and the second treatment consisted of exposing seed to 2.0 Krads of unmoderated fission neutrons. Further details of the procedure are given in the article by Constantin et al. (11). Eighteen rows of third generation progeny (M_3) were observed in the field during 1975 to be segregating for sterility. Seed were harvested from fertile plants and from sterile plants were seed existed. In general, sterile plants had no seed but occasionally a sterile plant had one or a few single-seeded pods. As a starting point in identification, it was assumed that the mutant was male-sterile. Seed were obtained from the Regional Soybean Laboratory at Urbana, Illinois, of ms_1 and ms_2 maintainer lines, T260H and T259H respectively. During the summer of 1978, crosses were made between the two maintainer lines and progeny of two mutant lines M_3-75-5298 and M_3-75-2111, segregating for sterility. M_3-75-5298 was a M_3 progeny row of D68-127 resulting from treatment with unmoderated fission neutrons.

M_3-75-2111 was a M_3 progeny row of D68-127 resulting from treatment with DES radiation.

Crosses were made between T260H and both D68-127 sources and between T259H and both D68-127 sources. At the onset of flowering pollen stains were made to identify sterile plants and heterozygous plants to use in making crosses. The stain used was lactophenol in cotton blue. The lactophenol was prepared by mixing 20 ml. phenol, 40 ml. glycerin and 40 ml. water. One to five ml. of 1% aqueous solution of cotton blue was added to the lactophenol to bring the stain to the desired intensity. Fertile pollen grains stained dark blue while sterile grains generally did not stain or stained very faintly and appeared empty.

F_1 seed were harvested in the fall and planted in plastic pots in the greenhouse. A single seed was planted in each pot. The pots were 28 centimeters in diameter and contained approximately 4500 grams of a 2:1:1 mix of soil, peat and perlite respectively. The pH and fertility of the mixture were adjusted to optimum level for plant growth.

In the spring of 1979 F_2 seed were harvested from each F_1 plant in the greenhouse and packaged for planting. The F_2 generation was planted in early June 1979 at The University of Tennessee Plant Sciences Field Laboratory located at Knoxville. Rows were three meters long with 40 seed per row. During September and early October plants of the F_2 generation were classified as either fertile or sterile. Classification was made after the pods and seeds had fully developed (R6 stage).

Plants which set normal appearing pods and seed were classified as fertile. Plants which had no pods or those which had clusters of vestigial pods were classified as sterile. Observed ratios were compared to expected ratios by use of chi-square.

CHAPTER III

RESULTS AND DISCUSSION

Preliminary tests involving D68-127 lines segregating for sterility were conducted at The University of Tennessee's Plant Sciences Field Laboratory at Knoxville in 1977. Large M_4 populations were rated at maturity for fertility and sterility. In almost all cases the result was a very good fit to a 3 fertile: 1 sterile ratio indicating that the sterility was conditioned by a single recessive gene. A point in favor of the gene involved conditioning male-sterility was that the sterile plants of D68-127 had the phenotypic characteristics normally associated with male-sterility. Throughout the preliminary tests and this study the sterile plants failed to mature normally, produced none to several pods, most of which were of the fleshy one-seeded type, and possessed dark green leathery leaves which remained green until frost. It was observed, however, during this study that many of the sterile plants produced more seed than normally associated with male-sterile plants.

A second point in favor of the gene conditioning male-sterility was the observed differences in pollen stainings of fertile and sterile plants. A large percentage of the pollen of sterile plants failed to stain or stained very lightly, whereas, pollen of fertile plants stained very heavily. Small and shrunken pollen grains were also observed where steriles were involved. Although these differences in pollen between fertile and sterile plants do not eliminate the

possibility of female sterility being involved it does help establish the presence of male-sterility in the material.

A final argument for male-sterility is that no seed were produced on either of the sterile F_1 plants in the greenhouse. This was probably due to the lack of pollen vectors. In the field many sterile plants produced at least one and in some cases several seed. If the sterility of D68-127 is the result of female abnormalities the lack of vectors in the greenhouse versus the field environment should have had no effect on pollination and seed production on sterile plants.

The failure of 87 of 114 F_1 seed to germinate in the greenhouse eliminated many hybrid combinations obtained for this study, thereby restricting the amount and type of combinations available for examination. Due to this loss no comparisons were available where the same D68-127 source plant was used in crosses with both ms_1 and ms_2 maintainer lines. Such comparisons would have been valuable in providing a check on plants which had been assigned a genotype on the basis of pollen stains prior to making the cross.

Researchers have reported pollen staining to be a reliable means of identifying fertile and sterile plants (8, 10, 12, 18, 23, 25). However, it was observed in this study that several plants identified during the summer of 1978 as being heterozygotes on the basis of pollen stains, remained green after frost. Several of these plants possessed a larger number of seed than normally expected on male-sterile plants.

In two instances, plants of the source line, M_3-75-2111, were identified as heterozygotes on the basis of pollen stains and used as

the male parent in successful crosses with the ms_1 maintainer line. A check of plants in the field after maturity revealed that these two "heterozygotes" were green and had retained their leaves which is characteristic of sterile plants. One of these plants yielded 31 seed. Several other plants were also identified as "heterozygotes" and used as the female in successful crosses with ms_1 and ms_2. These plants also remained green after maturity indicating that they were sterile. One of these plants yielded 34 seed.

These observations raise several questions which make interpretation of the data difficult. These questions are:

1. Was the technique used to identify a plant's genotype reliable?
2. Assuming the technique to be reliable, were errors made in interpreting the results of the pollen stainings?
3. Is the practice of assigning sterility to plants that remain green after maturity accurate?
4. Assuming the above practice is accurate, are sterile plants producing viable pollen in some cases?

There are no apparent reasons to doubt the reliability of the staining technique used. Cotton blue in lactophenol is a standard stain for pollen fertility counts. Fertile grains stain blue to dark blue while sterile grains fail to stain or stain very lightly.

The possibility of errors in the interpretation of the stain results does exist. Most of the difficulty encountered with stainings involved whether to assign a plant a heterozygous fertile or homozygous dominant fertile genotype. Generally the stain provided a clear

distinction between fertile and sterile plants. The conflict between the stain results and field results involved the distinction between heterozygous fertile and homozygous recessive sterile genotypes. Sterile plants possessed a few fertile pollen grains surrounded by many sterile grains. Plants classified as heterozygotes contained both fertile and sterile pollen grains but generally not in the expected 1:1 ratio. Instead fertile grains were predominant. The difficulty in distinguishing between heterozygous fertile and homozygous dominant fertile was due to the presence of some sterile pollen grains in almost every plant examined.

There is no evidence to doubt that plants that remain green after maturity are sterile. This is the standard procedure for classification of sterility. However, it was observed during the study that some plants remaining green after maturity had no pods while some had a few pods. Furthermore there were some "sterile" plants which had several pods, more than would be expected to result from cross-pollination. Assuming that all plants remaining green after maturity are sterile, the implication is that steriles do produce functional pollen in some instances. Hadley and Starnes (12) found that sterile plants of T241 produced 8.9% viable pollen grains and that sterile plants of T242 produced 6.2% viable pollen grains. Singh et al. (25) found that 30% of the pollen from Semmes M.S.1 and 35% of the pollen from ms_1 was stained by acetocarmine. Palmer (18) reported that 2.7% of the pollen from st_4 plants was stained by I_2KI. On two occasions during this study, sterile plants were incorrectly identified as heterozygotes

and used as the pollen parent in successful crosses. Therefore, the presence in some cases of viable pollen from steriles via pollen staining as well as seed production on sterile plants indicates that some of the steriles involved in this study produced viable pollen.

It seems that the most likely place for errors to have occurred in classification would have been in interpreting the results of the pollen staining. Therefore, the analysis will proceed with the assumption that the ratings made after maturity are the correct ones.

The following discussion of the results will be presented in two parts. The first part will compare the observed to expected ratios assuming that only one gene is involved in the sterility of D68-127 and that this gene is the same as ms_1. The second part will compare the observed to expected ratios assuming that only one gene is involved in the sterility of D68-127 and that the gene is the same as ms_2.

I. Ms_1 HYPOTHESIS

Heterozygote/Heterozygote Crosses

Assuming the gene causing sterility in D68-127 to be ms_1, crosses between heterozygotes of D68-127 and ms_1 would be expected to yield a ratio of 3 fertile: 1 sterile in the F_1 generation. The F_1s of the seven crosses of this genotypic combination were all fertile (Table 1). The lack of any steriles in the F_1 generation could be attributed to the low numbers involved. The probability of all seven F_1 plants being fertile is 13.3%.

Table 1. Observed and Expected F_1 Ratios for Different Genotypic Combinations.

Type of Cross	Proposed Genotype	Observed Ratio	Expected Ratios Hypothesis	
			ms_1	ms_2
ms_1/D68-127	$Ms_1ms_1Ms_2Ms_2/Ms_1ms_1Ms_2Ms_2$ $Ms_1ms_1Ms_2Ms_2/Ms_1Ms_1Ms_2ms_2$	7:0	3:1	1:0
ms_2/D68-127	$Ms_1Ms_1Ms_2ms_2/Ms_1ms_1Ms_2Ms_2$ $Ms_1Ms_1Ms_2ms_2/Ms_1Ms_1Ms_2ms_2$	15:2	1:0	3:1
ms_1/D68-127	$Ms_1ms_1Ms_2Ms_2/Ms_1Ms_1Ms_2Ms_2$ $Ms_1ms_1Ms_2Ms_2/Ms_1Ms_1Ms_2ms_2$	2:0	1:0	1:0
ms_1/D68-127	$ms_1ms_1Ms_2Ms_2/Ms_1ms_1Ms_2Ms_2$ $ms_1ms_1Ms_2Ms_2/Ms_1Ms_1Ms_2ms_2$	5:0	1:1	1:0
ms_2/D68-127	$Ms_1Ms_1ms_2ms_2/Ms_1ms_1Ms_2Ms_2$ $Ms_1Ms_1ms_2ms_2/Ms_1Ms_1Ms_2ms_2$	3:0	1:0	1:1

Thirty-three percent of the fertile F_1 plants would be expected to produce all fertile progeny (1:0) in the F_2 generation while the remaining 66% would be expected to segregate 3 fertile: 1 sterile. The observed and expected ratios and the chi-square values for these crosses are given in Table 2. Two of the seven crosses (28.6%) produced all fertile progeny (1:0) in the F_2 generation. The five segregating families (71.4%) fit the expected 3 fertile: 1 sterile ratio both individually and when pooled together (P = .25-.50). The total chi-square value indicates that a deviation as large or larger than observed would be expected between 10% and 25% of the time due to chance alone. The test for heterogeneity shows that a deviation as large as observed would be expected from a homogeneous population between 10% and 25% of the time due to chance alone. These data seem to lend support to the hypothesis that the gene involved in the sterility of D68-127 is ms_1.

Continuing under this hypothesis, crosses between heterozygotes of ms_2 and D68-127 will be considered. The result of these crosses are summarized in Table 3. Crosses of this genotypic combination would be expected to yield only fertile F_1 plants. This was found to be the case in 15 out of 17 crosses. Two F_1s were found to be sterile. There was some question, however, as to whether these steriles were the result of cross-pollinations or self-pollinations. The cross 15-14/7-2 was originally classified as a fertile/heterozygote. This particular combination was represented by three F_1 progeny. When advanced to the F_2 generation one of the families was found to be

Table 2. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Heterozygotes of ms_1 and D68-127, Assuming the Gene To Be ms_1.

Cross	Proposed Genotype	Observed Ratio	Expected Ratio 33% 1:0 66% 3:1	χ^2	$P > \chi^2$
1-11/6-2	$Ms_1ms_1Ms_2Ms_2/Ms_1ms_1Ms_2Ms_2$	281:115	3:1	3.448	.05-.10
1-16/6-3	" "	231:87	3:1	.943	.25-.50
1-6/14-21	" "	All Fertile	1:0	-----	-------
1-25/6-2	" "	193:49	3:1	2.915	.05-.10
1-25/6-2	" "	295:96	3:1	.042	.75-.90
2-9/6-2	" "	286:104	3:1	.578	.25-.50
2-13/4-7	" "	All Fertile	1:0	-----	-------
	(5df) Totals	1286:451		7.926	.10-.25
	(1df) Pooled			.861	.25-.50
	(4df) Heterogeneity			7.065	.10-.25

the result of a self-pollination and one was a cross and segregated 3 fertile: 1 sterile. The remaining F_1 was sterile and produced no seed. If this sterile was the result of a cross-pollination then the original classification was incorrect and the correct classification is heterozygote/heterozygote. The second sterile, from the cross 18-6/5-3, was originally classified as a heterozygote/heterozygote. This particular combination was represented by two F_1 progeny. When the fertile F_1 of this combination was advanced to the F_2 generation it was found to be the result of a self-pollination. Assuming that these steriles actually did result from cross-pollinations then it tends to disprove the present hypothesis that the gene causing sterility in D68-127 is ms_1. Steriles could arise in the F_1 only if both parents were segregating for the same sterility gene. This tends to support the alternate hypothesis that the gene causing sterility in D68-127 is ms_2. However, the possibility that one or both of the steriles were the result of self-pollinations cannot be disregarded.

Under the ms_1 hypothesis it would be expected that in the F_2 generation of this series of crosses 25% of the families would remain fertile (1:0); 50% of the families would segregate 3 fertile: 1 sterile and 25% of the families would segregate 9 fertile: 7 fertile. Five (33%) of the families were all fertile (1:0) in the F_2 generation (Table 3). Nine (60%) of the remaining ten families segregated in a 3 fertile: 1 sterile ratio. No families were observed that segregated 9 fertile: 7 sterile. One cross segregated in a manner that did not

Table 3. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Heterozygotes of ms_2 and D68-127, Assuming the Gene To Be ms_1.

Cross	Proposed Genotype	Observed Ratio	Expected Ratios 25% 1:0 50% 3:1 25% 9:7	χ^2	$P > \chi^2$
15-3/7-2	$Ms_1Ms_1Ms_2ms_2/Ms_1ms_1Ms_2Ms_2$	169:83†	3:1	8.465**	<.005
15-3/7-2	" "	153:48	3:1	.134	.50-.75
15-13/12-1	" "	All Fertile	1:0	-----	-------
16-4/7-2	" "	All Fertile	1:0	-----	-------
16-4/7-2	" "	All Fertile	1:0	-----	-------
16-4/7-2	" "	156:56	3:1	.226	.50-.75
16-10/10-18	" "	205:82	3:1	1.952	.10-.25
16-10/10-18	" "	161:54	3:1	.002	>.90
16-12/7-21	" "	276:107	3:1	1.762	.10-.25
16-12/7-21	" "	351:137	3:1	2.000	.10-.25
16-2/7-2	" "	285:94	3:1	.008	>.90
18-2/309	" "	250:84	3:1	.004	>.90
18-2/309	" "	All Fertile	1:0	-----	-------
18-2/4-6	" "	All Fertile	1:0	-----	-------
15-14/7-2	" "	282:92	3:1	.032	.75-.90
	(9df) Totals	2119:754		6.120	.50-.75
	(1df) Pooled			2.373	.10-.25
	(8df) Heterogeneity			3.747	.75-.90

**Significant at the .01 probability level.

†Not included in totals.

fit either of the expected ratios. The lack of any 9 fertile: 7 sterile ratios and the occurrence of two sterile in the F_1 generation seems to cast serious doubt on the ms_1 hypothesis. On the other hand, if the observed steriles in the F_1 generation resulted from self-pollinations then only the lack of 9:7 ratios disagrees with the ms_1 hypothesis. The test for heterogeneity indicates that all the progeny are from a population that is segregating 3 fertile: 1 sterile.

Fertile/Heterozygote Crosses

The results of crosses between heterozygotes of ms_1 and fertiles D68-127 are presented in Table 4. Assuming the gene causing sterility in D68-127 to be ms_1 this genotypic combination would be expected to produce only fertile F_1 plants. This was found to be the case (Table 1, page 19). In the F_2 generation 50% of the families would be expected to remain fertile (1:0) and 50% of the families would be expected to segregate 3 fertile: 1 sterile. Both of the families remained fertile in the F_2 generation (Table 4). However, since the numbers were so low, this does not represent a significant departure from the expected and would agree with the ms_1 hypothesis.

Sterile/Heterozygote Crosses

The results of crosses between steriles and heterozygotes of ms_1 and D68-127 are presented in Table 5. The expected F_1 ratio for the genotypic combination would be 1 fertile: 1 sterile. All five F_1s were fertile (Table 1, page 19). The probability of all five being fertile where a 1:1 ratio is involved is 3.1%. The expected F_2 ratio

Table 4. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Fertiles and Heterozygotes of ms_1 and Fertiles of D68-127, Assuming the Gene To Be ms_1.

Cross	Proposed Genotype	Observed Ratios	Expected Ratios 50% 1:0 50% 3:1	χ^2	$P > \chi^2$
1-21/6-4	$Ms_1ms_1Ms_2Ms_2/Ms_1Ms_1Ms_2Ms_2$	All Fertile	1:0	-----	-------
1-21/6-4	$Ms_1ms_1Ms_1Ms_2/Ms_1Ms_1Ms_2Ms_2$	All Fertile	1:0	-----	-------

Table 5. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Steriles and Heterozygotes of ms_1 and D68-127, Assuming the Gene To Be ms_1.

Cross	Proposed Genotype	Observed Ratios	Expected Ratio 100% 3:1	χ^2	$P > \chi^2$
1-8/8-3	$ms_1ms_1Ms_2Ms_2/Ms_1ms_1Ms_2Ms_2$	230:58	3:1	3.629	.05-.10
6-20/1-11	" "	95:30	3:1	.007	.75-.90
6-8/2-8	" "	152:48	3:1	.107	.50-.75
1-11/6-8	$Ms_1ms_1Ms_2Ms_2/ms_1ms_1Ms_2Ms_2$	247:70	3:1	1.439	.10-.25
		274:91	3:1	.001	.90
	(5df) Totals	998:297		5.183	.25-.50
	(1df) Pooled			2.947	.05-.10
	(4df) Heterogeneity			2.236	.50-.75

of all fertile F_1s would be 3 fertile: 1 sterile. It can be seen from Table 5 that all five families fit ($P \geq .05$) a 3 fertile: 1 sterile ratio. The five families fit a 3 fertile: 1 sterile ratio both individually and as a group. The total chi-square value indicates that a deviation as large as observed would be expected between 25% and 50% of the time. The test for heterogeneity indicates that all five families are from a population that is segregating 3 fertile: 1 sterile.

It can be seen from Table 5 that in two instances sterile plants were used as the pollen parent. This point was alluded to at the beginning of this chapter. Plants 6-8 and 6-20 were originally classified as heterozygous and were used as male parents in crosses. At maturity, however, these plants remained green and therefore must be considered to be sterile. If the classification of green plants at maturity as being steriles is a correct one, then it is apparent that sterile plants do in some instances produce viable pollen. This seems to be contrary to present beliefs since ms_1 supposedly produces nonfunctioning pollen while ms_2 is said to produce no pollen. However, singh et al. (25) observed that up to 35% of the pollen from ms_1 plants was stained by acetocarmine. This could help explain the mistake in classification of plants 6-8 and 6-20 since a large percentage of stained pollen would place them in the heterozygote class. Differences in the amount of viable pollen among sterile plants could also help explain observed differences in seed set among sterile plants in the same environment.

The results of crosses between steriles and heterozygotes of ms_2 and D68-127 are presented in Table 6. All F_1 plants of this genotypic combination would be expected to be fertile, as was observed (Table 1, page 19). In the F_2 generation of these crosses 50% of the families would be expected to segregate 3 fertile: 1 sterile and 50% would be expected to segregate 9 fertile: 7 sterile, under the ms_1 hypothesis. Two of the three families segregated in a manner that gave a very poor fit to a 9 fertile: 7 sterile ratio. The remaining cross segregated in a manner that failed to fit either a 3 fertile: 1 sterile or a 9 fertile: 7 sterile ratio, however, this cross did come closer to fitting a 3 fertile: 1 sterile ratio. It is tempting to conclude that two of the families actually represent a 9 fertile: 7 sterile population and that the third family actually represents a 3 fertile: 1 sterile population and that the large chi-square values are due to chance variations. If this were the case then this series of crosses would agree very well with the ms_1 hypothesis. However, this cannot be concluded from the data. At best it can be said that two of the families seem to fit a 9 fertile: 7 sterile ratio very poorly and that the remaining cross comes close to giving a very poor fit to a 3 fertile: 1 sterile ratio. Thus, this series of crosses does not provide any strong evidence to either prove or disprove the ms_1 hypothesis.

Table 6. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Steriles and Heterozygotes of ms_2 and D68-127, Assuming the Gene To Be ms_1.

Cross	Proposed Genotype	Observed Ratios	Expected Ratios 50% 3:1 50% 9:7	χ^2	$P > \chi^2$	Expected Ratios 50% 3:1 50% 9:7	χ^2	$P > \chi^2$
15-9/7-2	$Ms_1Ms_1ms_2ms_2/Ms_1ms_1Ms_2Ms_2$	142:79		13.612**	<.005	9:7	5.752*	.01-.025
15-12/7-2	" "	152:79		10.426**	<.005	9:7	8.563*	.01-.025
15-12/7-2	" "	232:37†		------	------	9:7	-----	--------
	(2df) Totals	294:158		24.038**	<.005		14.315**	<.005
	(1df) Pooled			23.894**	<.005		14.203**	<.005
	(1df) Heterogeneity			.144	.50-.75		.112	.50-.75

**Significant at the 0.05 and 0.01 probability levels, respectively.

†Not included in totals.

II. Ms_2 HYPOTHESIS

Heterozygote/Heterozygote Crosses

Assuming the gene causing sterility in D68-127 to be ms_2, crosses between heterozygotes of D68-127 and ms_1 would be expected to yield only fertile plants in the F_1 generation. This was found to be true for the seven crosses of this genotypic combination (Table 1, page 19). In the F_2 generation 25% of the families would be expected to be fertile (1:0); 50% would be expected to segregate 3 fertile: 1 sterile and 25% would be expected to segregate 9 fertile: 7 sterile. The observed ratios, expected ratios and chi-square values for these crosses are given in Table 7. Two (28.6%) of the seven F_2 families were completely fertile and the remaining five (71.4%) families segregated in a manner that apparently fits a 3 fertile: 1 sterile ratio. No 9 fertile: 7 sterile ratios were observed. The lack of any 9 fertile: 7 sterile ratios could possibly be explained by the low numbers involved. Only 25% of the families are expected to segregate 9 fertile: 7 sterile. With seven families, approximately two would be expected to segregate 9 fertile: 7 sterile. The total chi-square value indicates that a deviation from a 3 fertile: 1 sterile ratio as large as that observed would be expected between 10% and 25% of the time. The pooled chi-square value gives a good fit to a 3 fertile: 1 sterile ratio. The test for heterogeneity indicates that all five of the segregating families could be from a population segregating in a 3 fertile: 1 sterile ratio. These results seem to be consistent with the ms_2 hypothesis.

Table 7. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Heterozygotes of ms_1 and D68-127, Assuming the Gene To Be ms_2.

Cross	Proposed Genotype	Observed Ratios	Expected Ratios 25% 1:0 50% 3:1 25% 9:7	χ^2	$P > \chi^2$
1-11/6-2	$Ms_1ms_1Ms_2Ms_2/Ms_1Ms_1Ms_2ms_2$	281:115	3:1	3.448	.05-.10
1-16/6-3	" "	231:87	3:1	.943	.25-.50
1-6/14-21	" "	All Fertile	1:0	-----	-------
1-25/6-2	" "	193:49	3:1	2.915	.05-.10
1-25/6-2	" "	295:96	3:1	.042	.75-.90
2-9/6-2	" "	286:104	3:1	.578	.25-.50
2-13/4-7	" "	All Fertile	1:0	-----	-------
	(5df) Totals	1286:451		7.926	.10-.25
	(1df) Pooled			.861	.25-.50
	(4df) Heterogeneity			7.065	.10-.25

Under the ms_2 hypothesis, crosses between heterozygotes of ms_2 and D68-127 would be expected to result in a 3 fertile: 1 sterile ratio in the F_1 generation. The actual ratio observed was 15 fertile: 2 sterile (Table 1, page 19). The expected numbers would be 12.75 fertile: 4.25 sterile out of a total of 17. The two sterile F_1 plants are again a point of concern, as it was in the discussion of the ms_1 hypothesis. The question is whether or not they resulted from cross-pollinations or self-pollinations. In the F_2 generation of this genotypic combination 33% of the families would be expected to be fertile (1:0) and 66% of the families would be expected to segregate in a 3 fertile: 1 sterile ratio. The observed ratios, expected ratios and chi-square values for this series of crosses are given in Table 8. Five (33%) of the families remained fertile in the F_2 generation. This represents a perfect fit to the expected ratio. Nine (60%) of the remaining ten families segregated in a manner that fit a 3 fertile: 1 sterile ratio. They fit a 3 fertile: 1 sterile ratio both individually and as a group. The total chi-square gave a very good fit to a 3 fertile: 1 sterile ratio. The test for heterogeneity indicates that the probability is between 75% and 90% that all nine families are from a population that is segregating in a 3 fertile: 1 sterile ratio. One of the ten segregating families gave a very poor fit to a 3 fertile: 1 sterile ratio. This could be the result of a chance deviation from a 3 fertile: 1 sterile ratio, or it could just as easily be a chance deviation from a 9 fertile: 7 sterile ratio in which case it would tend to disprove the ms_2 hypothesis. However,

Table 8. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Heterozygotes of ms_2 and D68-127, Assuming the Gene To Be ms_2.

Cross	Proposed Genotype	Observed Ratios	Expected Ratios 33% 1:0 66% 3:1	χ^2	$P > \chi^2$
15-3/7-2	$Ms_1Ms_1Ms_2ms_2/Ms_1Ms_1Ms_2ms_2$	169:83†	3:1	8.466**	<.005
15-3/7-2	" "	153:48	3:1	.134	.50-.75
15-13/12-1	" "	All Fertile	1:0	-----	-------
16-4/7-2	" "	All Fertile	1:0	-----	-------
16-4/7-2	" "	All Fertile	1:0	-----	-------
16-4/7-2	" "	156:56	3:1	.226	.50-.75
16-10/10-18	" "	205:82	3:1	1.952	.10-.25
16-10/10-18	" "	161:54	3:1	.002	>.90
16-12/7-21	" "	276:107	3:1	1.762	.10-.25
16-12/7-21	" "	351:137	3:1	2.000	.10-.25
16-2/7-2	" "	285:94	3:1	.008	>.90
18-2/3-9	" "	250:84	3:1	.004	>.90
18-2/3-9	" "	All Fertile	1:0	-----	-------
18-2/4-6	" "	All Fertile	1:0	-----	-------
15-14/7-2	" "	282:92	3:1	.032	.75-.90
	(9df) Totals	2119:754		6.120	.50-.75
	(1df) Pooled			2.373	.10-.25
	(8df) Heterogeneity			3.747	.75-.90

**Significant at the 0.01 probability level.

†Not included in totals.

this cross does come closer to fitting a 3 fertile: 1 sterile ratio than it does to a 9 fertile: 7 sterile ratio. Assuming that the sterile F_1s did result from cross-pollinations instead of self-pollinations the results agree very closely with the ms_2 hypothesis. Sterile plants could have resulted in the F_1 generation only if both parents were segregating for the same sterility gene, in this case ms_2. The two sterile F_1s along with the relatively good fit of the observed to the expected ratios in this series of crosses lends strong support to the ms_2 hypothesis. On the other hand, if the sterile F_1s resulted from self-pollinations much of the evidence for the ms_2 hypothesis is lost. A 3 fertile: 1 sterile ratio was expected in the F_1 generation of this series of crosses. The two steriles in question were the only ones observed out of 17 total F_1 plants. If these steriles were actually the result of self-pollinations then no steriles were observed out of 15 total F_1 plants. The probability of not recovering any steriles out of 15 chances when a 3 fertile: 1 sterile ratio is involved is 1.3%. The probability of recovering only 1 sterile out of 15 is 6.7%. However, the observed segregation in the F_2 generation compares very well with the expected regardless of how the sterile F_1s arose. So depending on whether the sterile F_1s are assumed to have resulted from cross-pollinations or self-pollinations this series of crosses may or may not offer good evidence for the ms_2 hypothesis.

Sterile/Heterozygote Crosses

The results of crosses between steriles and heterozygotes of ms_1 and D68-127 are presented in Table 9. Under the ms_2 hypothesis

Table 9. Observed Ratios, Expected Ratios and Chi-Square Values for Crosses between Steriles and Heterozygotes of ms_1 and D68-127, Assuming the Gene To Be ms_2.

Cross	Proposed Genotype	Observed Ratios	Expected Ratios 50% 3:1 50% 9:7	χ^2	$P > \chi^2$
1-8/8-3	$ms_1ms_1Ms_2Ms_2/Ms_1Ms_1Ms_2ms_2$	230:58	3:1	3.629	.05-.10
6-20/1-11	$Ms_1Ms_1ms_2ms_2/Ms_1ms_1Ms_2Ms_2$	95:30	3:1	.007	.75-.90
6-8/2-8	" "	152:48	3:1	.107	.50-.75
1-11/6-8	$Ms_1ms_1Ms_2Ms_2/Ms_1Ms_1ms_2ms_2$	247:70	3:1	1.439	.10-.25
1-11/6-20	" "	274:91	3:1	.001	>.90
	(4df) Totals	998:297		5.183	.25-.50
	(1df) Pooled			2.949	.05-.10
	(4df) Heterogeneity			2.236	.50-.75

this genotypic combination would be expected to produce only fertile F_1 plants, as was observed (Table 1, page 19). In the F_2 generation 50% of the families would be expected to segregate in a 3 fertile: 1 sterile ratio and 50% of the families would be expected to segregate in a 9 fertile: 7 sterile ratio. All five families fit a 3 fertile: 1 sterile ratio both individually and when pooled together. The probability of this occurring due to chance alone is 23.7%. The test for heterogeneity indicates that all five families are from a population that is segregating in a 3 fertile: 1 sterile ratio. Again it can be seen from Table 9 that sterile plants, as classified at maturity were used as the male parent in successful crosses indicating that sterile plants do produce viable pollen in some instances.

The results of crosses between steriles and heterozygotes of ms_2 and D68-127 are presented in Table 10. Under the ms_2 hypothesis the F_1 generation of this genotypic combination would be expected to segregate in a 1 fertile: 1 sterile ratio. All three F_1 plants were fertile (Table 1, page 19). The probability of all three F_1 plants being fertile when a 1:1 ratio is involved is 12.5%. The lack of sterile F_1 plants could possibly be explained by the low numbers involved in this particular combination. The F_2 generation would be expected to segregate in a 3 fertile: 1 sterile ratio in all cases. Two of the three families fit a 9 fertile: 7 sterile ratio very poorly. The remaining cross did not fit a 3 fertile: 1 sterile nor a 9 fertile: 7 sterile ratio. However, it came closer to giving a poor fit to a 3 fertile: 1 sterile ratio. No valid conclusions can

be drawn from these data since the segregation patterns do not fit either of the expected ratios. However, it would appear that two of the three families may be segregating in a 9 fertile: 7 sterile ratio, thereby, providing evidence against the ms_2 hypothesis.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The objective of this study was to determine if the sterility mutant of D68-127 was a male-sterile mutant conditioned by either the ms_1 or ms_2 gene for male-sterility. The conclusions that can be drawn are:

1. It appears that the gene involved in the sterility of D68-127 is a single recessive gene and that it is male-sterile.

2. The data obtained from this study best support the ms_1 hypothesis, however, the possibility that it is ms_2 or ms_n cannot be ruled out.

3. Sterile plants, or those remaining green at maturity, of ms_1, ms_2 and D68-127 mutant produced variable amounts of viable pollen which resulted in seed production in many instances, indicating that none of the genes provided complete male-sterility.

The F_2 ratios, although not giving any conclusive evidence as to the exact gene involved support the hypothesis that the sterility of D68-127 is male-sterility conditioned by a single recessive gene. First 19 of 23 segregating F_2 families fit a 3 fertile: 1 sterile ratio which is the expected ratio for a single recessive gene segregating in the F_2 generation. Secondly, sterile plants of D68-127 exhibited the phenotypic characteristics discussed for ms_1 and ms_2 earlier in the review of literature and observed in the field during this study. Circumstantial evidence for male-sterility was the observed differences

in pollen stainings of viable and nonviable pollen of fertile versus sterile plants of D68-127 mutant.

The absence of any 9 fertile: 7 sterile ratios in the F_2 generation prevents a conclusion from being drawn regarding the exact gene involved in the sterility of D68-127. However, the F_2 ratios of three of the five types of crosses listed in Table 1 (page 19) lend support to the ms_1 hypothesis. Crosses between fertiles and heterozygotes of ms_1/D68-127 support either gene hypothesis equally well. This being due to the fact that the expected ratios for this genotypic combination are the same regardless of the gene involved. On the other hand, the F_2 ratios of crosses between heterozygotes of ms_2/D68-127 support the ms_2 hypothesis. The only conclusion that can be made is that the data obtained from this study best support the ms_1 hypothesis. It should be pointed out, however, that in four of the five types of crosses the number of families involved was relatively low.

When this study began ms_3 and ms_4 had not been discovered and were not included in the allelism tests. Therefore, any conclusions regarding the gene involved in the sterility of D68-127 must be restricted to ms_1 and ms_2 while keeping in mind that the expected ratios would be the same, provided that the sterility is due to one gene as proposed, regardless of whether the gene is ms_1, ms_2, ms_3, ms_4 or ms_n.

Based on the results of this study it can be concluded that sterile plants of the D68-127 mutant produce viable pollen in some cases as evidenced by successful crosses where sterile plants were

used as the male parent. Additional evidence for this conclusion was seen in the results of the pollen stainings in which some sterile plants were observed to produce low numbers of viable appearing pollen grains. This observed difference in pollen staining among sterile plants could explain the large differences in seed production among sterile plants. These observations parallel those for "sterile" plants from ms_1 and ms_2 maintainer lines. For example some sterile plants of ms_1 and ms_2 produced as many as 72 and 40 pods, respectively when grown in the field at Knoxville during 1979. The number of pods and seeds produced were more than can be realistically explained on the basis of cross-pollinations.

Steriles of ms_1, ms_2 and D68-127 mutant and those arising from crosses between D68-127 and ms_1 and ms_2 showed a large amount of variation in seed production under the same environmental conditions. Regardless of the gene involved, none imparted complete male sterility to their so-called sterile progeny. Instead, many of the steriles resembled what one would expect from a gene conditioning partial male-sterility.

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LITERATURE CITED

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