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I am submitting herewith a dissertation written by Thomas Edward Morgan entitled "Genetics and Inheritance of Fertility Restoration of Several Male-Sterile Cytoplasms in Corn (Zea mays L.)." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Science.

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GENETICS AND INHERITANCE OF FERTILITY RESTORATION OF SEVERAL MALE-STERILE CYTOPLASMS IN CORN (ZEA MAYS L.)

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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ABSTRACT

There has been limited use of male-sterile cytoplasm in the production of hybrid seed corn (Zea mays L.) since the 1970 epiphytotic of southern corn leaf blight (Helminthosporium maydis), race T. Corn researchers have since been studying the characteristics of other male-sterile cytoplasms in an attempt to replace the Texas source. Information regarding the inheritance of fertility restoration of these cytoplasms is essential in evaluating their potential usefulness.

The inheritance of fertility restoration of several male-sterile cytoplasms was evaluated from fertility data of individual plants of F_1, F_2, and backcross generations. Inbreds Ky21 and T115 were used as restorer lines. The effect of planting date on fertility restoration of the various male-sterile cytoplasms also was determined.

Restorers Ky21 and T115 fully restored the fertility of sterilized lines containing T, SC, and S-group male-sterile cytoplasms, but only partially restored the fertility in C, RB, and El Sal cytoplasms.

Cytoplasms T and SC reacted similarly in fertility restoration and appear to belong to the same group. The fertility restoration of most S-group cytoplasms was similar when crossed by T115, but variations among these cytoplasms were observed when crossed by Ky21.

Fertility data obtained from crosses containing C, RB, and El Sal cytoplasms were too variable to determine the inheritance of their fertility restoration. These cytoplasms appear, however, to belong to the same group.

Date of planting had little effect on fertility restoration of T, SC, and S-group cytoplasms. The fertility expression of C, RB, and El Sal cytoplasms was greatly affected by date of planting, with fertility restoration being inhibited more in the later plantings.

The results of this study suggest that since restoring and nonrestoring inbreds differ in genotype with respect to fertility restoration, any statement concerning the fertility restoration of a certain male-sterile cytoplasm is true only with respect to the particular restorer and sterilized lines used in the cross.

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CHAPTER I

INTRODUCTION

A major problem confronting early hybrid seed corn (Zea mays L.) producers was the expensive task of removing the tassels from female parents in crossing fields. It appeared that the labor and expense involved in detasseling might become a serious obstacle to large-scale production and widespread use of hybrid seed. Thus, corn researchers began intensifying their investigations of alternative methods to circumvent the task of detasseling. Methods such as separating selfed and crossed seed by specific gravity and endosperm color techniques, the use of chemicals to prevent pollen production, and the application of genetic male sterility were investigated, but all were found to be less than satisfactory (28).

A major advance, however, in eliminating the detasseling procedure in hybrid corn production came about in the early 1950's with the development and commercial use of cytoplasmic male sterility (13,30, 37). Through the use of cytoplasmic male sterility and specific fertility restoring genes, hybrid corn producers were able to produce hybrid seed without detasseling the female parent (29,37). This plant breeding achievement brought about a revolutionary change in the hybrid corn industry.

Several sources of cytoplasmic male sterility in corn were known and had been studied, but the Texas (T) source was found to be more

suitable for hybrid seed production due to its greater stability and reliability of fertility restoration. By 1970, T cytoplasm was being used to produce about 85 percent of the hybrid seed corn in the United States (43). An epiphytotic outbreak of southern corn leaf blight caused by Helminthosporium maydis, race T, occurred in 1970. This race was highly specific in pathogenicity to inbreds and hybrid plants with T cytoplasm. Since a majority of the corn grown in the United States contained T cytoplasm, the disease ran rampant across the country. Damage from this one disease totaled nearly one billion dollars nationwide with one-fourth of the corn crop destroyed in the state of Illinois (2).

After the 1970 southern corn leaf blight epiphytotic, the use of T cytoplasm was virtually eliminated from hybrid seed production; and producers had to return to the use of normal cytoplasm along with the process of hand or mechanical detasseling. In recent years plant breeders have begun to look into the potential usefulness of other male-sterile cytoplasms to replace the T source (15).

The objectives of this study were: (1) to determine whether fertility in various male-sterile cytoplasms other than T is restored by the universal restoring inbreds Ky21 and T115, (2) to determine whether the inheritance of fertility restoration in these other cytoplasms by the universal restorers Ky21 and T115 is similar to that in T cytoplasm, (3) to characterize the various sources of male-sterile cytoplasms, and (4) to determine the effect of planting date on fertility restoration of various male-sterile cytoplasms.

CHAPTER II

REVIEW OF LITERATURE

The first occurrence of cytoplasmic inheritance of male sterility in corn was reported by Rhoades (35) in 1931. One male-sterile plant was observed in the offspring from an open-pollinated ear of corn collected by R. A. Emerson and F. D. Richey at Arequipa, Peru. The male-sterile plant crossed to a Chilean variety produced an F_1 generation which was completely male-sterile. From further testing, Rhoades (35) concluded that the male sterility was due entirely to factors contributed by the female parent. This Peruvian source of cytoplasmic sterility was later lost.

A second case of cytoplasmic male sterility in corn was reported by Gini (cited by Edwardson, 19) in Argentina in 1939. An interaction of nuclear genes with a specific cytoplasm appeared necessary to produce the male sterility effect, since, depending on the male parent, progenies either segregated for pollen sterility or were completely male sterile. This source of cytoplasmic sterility also has been lost.

Josephson and Jenkins (31) examined male sterility in certain hybrids after farmers in Kentucky, Tennessee, and Indiana reported fields which failed to set seed properly in the season of 1945. Examination of the hybrid pedigrees, followed by a series of special crosses, clearly showed that the inbred 33-16 carried a cytoplasm which caused pollen sterility to be exhibited, providing its own germ plasm

was replaced or combined with certain other genotypes (30). This male-sterile cytoplasm was given the source name of J.

The USDA or S source of male-sterile cytoplasm was first described by Jones et al. (29). This source of pollen sterility was observed in a progeny grown by M. T. Jenkins, of the United States Department of Agriculture. Jones and co-workers found that this source of cytoplasmic pollen sterility, like the ones previously described, was affected by genotype in its fertility expression. Some genotypes were sterile in this cytoplasm while others were partially or completely fertile.

The Texas source of cytoplasmic male sterility was discovered independently by Mangelsdorf (Jones and Mangelsdorf, 28) in the "Mexican June" variety and Rogers (Rogers and Edwardson, 37) in the variety, "Golden June." This source of pollen sterility also was affected in expression by the genotype.

Schwartz (38) described the occurrence of the "Kys" type of male sterility as resulting from the interaction of a specific sterile cytoplasm with two pairs of genetic factors. Leng and Bauman (34), however, studied the utilization of the "Kys" type of male sterility and reported that this type of male sterility was due only to nuclear genes and could not be employed successfully to produce hybrid seed. Efforts to incorporate the "Kys" type of sterility into inbred lines were then discontinued.

Many additional discoveries of male-sterile cytoplasms in corn have been described. In 1965, 84 such discoveries were noted (14), and since then, several more have been reported (4). The existence of

nuclear genes, usually dominant in action, which restore male fertility in spite of the presence of sterile cytoplasm, has provided the opportunity to characterize and group the various strains. The usual procedure is to cross the cytoplasmic male-sterile individuals with pollen from a series of established inbred lines and note the pattern of restoration among the offspring. Briggle (7) and Duvick (14) concluded from their studies that at least two cytoplasmic groups existed, those resembling S cytoplasm and those resembling T.

Beckett (4) analyzed a number of cytoplasmic male-sterile strains by crossing and backcrossing repeatedly with a series of inbred lines used as male parents. Thirty male-sterile strains, from a variety of independent sources, and eighteen inbreds were involved in the study. On the basis of the pattern of restoration, most of the male-sterile cytoplasms were assignable to either T or S groups. Two of the sources, however, C and RB, had restoration responses which fit neither the T nor the S pattern. The sterility-inducing cytoplasm which they carry has been designated the C group.

The S group in Beckett's (4) study included sources S, G, I, J, M, R, ML, VG, and probably sources F, H, K, L, W, CA, EK, IA, MY, PS, SD, and TA. The T group consisted of T, Q, HA, and probably P sources. Unclassified sources were B, D, and ME.

Studies by Gracen (26) and Gracen and Grogan (27) also established three main groups similar to the T, C, and S groups. They included source RS in the T group and ME in the S group. All other classifications were similar to Beckett's. Gracen (26), by studying the

fertility restoration reaction patterns of the cytoplasms in combination with several inbred lines, was able to detect variation within the fertility restoration reactions of certain cytoplasms previously classified in the S group. Cytoplasms CA, EK, G, K, L, ME, ML, PS, and TA, although similar to S in fertility restoration in some inbred backgrounds, could be differentiated from S cytoplasm in other inbreds. Groups C and S were all resistant to race T of H. maydis and to Phyllosticta maydis yellow leaf blight.

Complete restoration of pollen fertility in the T type of cytoplasmic male sterility of corn had been assumed by many authors to be due to the action of a single dominant gene. Edwardson (18) concluded from F_2 and backcross data that a single pair of genes was responsible for fertility restoration of T cytoplasm. Thomas and Johnson (42) and Rogers (36) studied the fertility restoring capabilities of inbreds K55 and Tx127c and concluded that the restoration of T cytoplasm was due to a single gene. Blickenstaff et al. (6) reported that pollen fertility restoration in T cytoplasm was due primarily to a single dominant gene located on chromosome 3.

Further investigations, however, showed that complete fertility restoration of T cytoplasm was due to at least two dominant genes. Duvick's (11,13,14) studies pointed out that restoration of T cytoplasm required the simultaneous presence of at least two dominant genes as well as some modifiers. The two major genes have been designated Rf_1, which is located on the proximal end of the long arm of chromosome 3 (6,16,44), and Rf_2, which is located on chromosome 9 approximately 5

crossover units from waxy endosperm (wx) and adjacent to the centromere (39). The Rf_2 gene is present in homozygous dominant form in nearly all types of corn (9,14,16,25), whereas, the Rf_1 restorer gene is generally absent.

Since most corn inbreds are on the genotype rf_1rf_1, Alexander and Beckett (1) proposed a search for a gene conversion allele Rf_1' that would transform rf_1 locus to Rf_1 and give immediate homozygosity for full restoration. The production of double-cross hybrids by use of the Eckhardt (17) method requires one Rf_1Rf_1 inbred if half the plants are to shed pollen and two such inbreds if all plants are to be fertile. The Rf_1' system would require only one fertility restoring inbred to produce fully restored double-cross hybrids.

Stinson (40) reported different numbers of genes involved in fertility restoration of T cytoplasm, depending upon the particular sterile inbred and restorer line used. From F_2 and backcross data, he observed genetic ratios indicating a single dominant gene for restoration, two dominant genes acting in a complementary way, and also duplicate dominant genes, either one of which could restore fertility. Duvick (11) obtained genetic ratios in a particular cross that indicated a single dominant gene plus three modifier genes responsible for complete restoration in T cytoplasm. Two of the three modifier genes were duplicates.

The major gene for fertility restoration in S cytoplasm has been assigned the symbol Rf_3 (8,14,24). Briggle (7) observed from data of S cytoplasm populations an indication of some type of selective

fertilization in plants that were heterozygous for the fertility restoring gene. Investigation of this type of behavior led Buchert (8) to discover a fundamental difference between the S and T cytoplasms. He concluded that in S cytoplasm only those microspores which contain the dominant gene for fertility restoration in S cytoplasm (Rf_3) will develop into fertile pollen grains; the microspores containing the recessive allele (rf_3) abort. In Texas cytoplasm, in contrast, all (95-100 percent) pollen grains in a plant are fertile if the plant is at least heterozygous for the dominant gene or genes necessary for fertility restoration. The net result of this phenomenon is to upset segregation ratios when the source of segregating gametes is pollen from plants with S cytoplasm which are heterozygous for the major restorer gene. Buchert (8), however, showed that segregation was normal if such plants were used as female or if Rf_3rf_3 plants in normal cytoplasm were used as either male or female.

Stinson (40) reported from observations of pollen restoration that the various restorers may differ in genotype with respect to restoration, as observed when different restorers were used on the same sterile inbred. He also found that different inbreds with the same source of male-sterile cytoplasm influenced differently the degree of restoration, as observed when various sterile inbreds were combined with the same restorer. Duvick (13) pointed out that not all sterilizable inbreds were recessive for the same genes, so that a restorer line which had sufficient dominants for one inbred might be insufficient for another.

The restoration of cytoplasmic male sterility is sometimes affected by environmental conditions. Duvick (11,13) reported that in some

environments, such as hot and dry conditions during flowering, the genes normally responsible for complete restoration of T cytoplasm were without full effect unless there were at least two dominant modifier genes present. Beckett (3) observed that partial restoration of T cytoplasm depends upon the action of genes located on several chromosomes. Generally, only partial restorer genotypes in T cytoplasm are affected by environment. Hot dry conditions at the time of flowering usually cause partial restorers to be more sterile, and cool humid conditions at this time cause partial restorers to have a higher percentage of fertile pollen (14).

Blickenstaff et al. (6) observed that long photoperiod and possibly high temperature had an inhibiting effect on fertility restoration of some inbreds. Duvick (14) found in the gametophytic controlled S cytoplasm that in some genotypes a small percentage of fertile pollen grains of rf_3 genotype are produced by Rf_3rf_3 plants, in addition to the fertile Rf_3 pollen grains. He concluded that it was not known whether specific modifying genes, or environmental conditions, or a combination of these factors govern this departure from the general rule.

Despite extensive knowledge and the large number of breeding programs exploiting cytoplasmically inherited male sterility, an understanding of the mechanism(s) of cytoplasmic male sterility is still lacking. The early attempts to describe the mechanism of cytoplasmic male sterility considered viruses or episomal-like particles, which could exist and replicate in the cytoplasm, as candidates for inactivating pollen, but no experiments really substantiated the

existence of any infectious agents (14,21). Edwardson (20) investigated differences between T and normal cytoplasm by electron microscopy. He found inclusions associated with dark areas in T cytoplasm as compared with none in the normal type.

Flavell (23) proposed a model to account for the features of cytoplasmically inherited male sterility in higher plants. He suggested that normal anther development is prevented by an interaction between a substance(s) present only in anther and organelles with altered structures. The alteration in the organelles (mitochondria or chloroplasts) results from mutations in mitochondria or chloroplast DNA. He postulated that nuclear fertility restorer genes correct the altered organelle structure, making the organelles insensitive to the anther substance(s) or interfere with the production or transport of the anther substance(s).

Morphological differences have been observed between normal and male-sterile plants. Jones and Mangelsdorf (28) found a slight shortening of plant height in sterile corn with T cytoplasm. A shortening of the stalk above the ear in some lines of corn was found. Josephson and Kincer (32) indicated that shortening was confined to the upper 2 or 3 internodes. Duvick (14) reported that T cytoplasm reduced the number of leaves per plant by 1-2 percent. From these studies it is concluded that, in leaf number as well as plant height, T cytoplasm affects the plant prior to meiosis and does so independently of its effect on pollen fertility.

Duvick (12) stated that cytoplasmic male-sterile hybrids were sometimes different in yield from their normal cytoplasm counterparts,

with extent and direction of difference depending on both the hybrid and environment. Everett (22) and Josephson and Kincer (32) concluded that the cytoplasm has little or no effect on yield. Stringfield (41) reported that T cytoplasm plus the restorer genotype might raise yields. Chinwuba et al. (10) found that pollen sterility, as such, relieves some of the stress on the plant at time of flowering, resulting in higher yields for at least some pollen-sterile hybrids as compared to their fertile counterparts whenever the hybrids have been under reasonably severe stress at the time of flowering. They reported that cytoplasmic male sterility and male sterility caused by detasseling are both effective.

Beckett (5) summarized the features of the different cytoplasmic groups. Full fertility restoration of T cytoplasm is under sporophytic control; i.e., the genetic constitution of the plant determines whether or not the pollen survives. Most genotypes are easily sterilized by Texas cytoplasm since few inbreds possess restorer genes. The phenotype of T cytoplasm is relatively stable under different environmental conditions with a relatively low mutation rate. Texas cytoplasm is highly susceptible to H. maydis, race T, and Phyllosticta maydis yellow leaf blight.

Fertility restoration of S cytoplasm, as summarized by Beckett (5), is under gametophytic control with only 50 percent viable pollen in hybrids. Few genotypes are sterilized by S cytoplasm since many inbreds contain weak restorer genes. The phenotype of S cytoplasm is relatively unstable with a relatively high mutation rate. Inbred WF9, as pointed

out by Beckett (5), frequently gives fertile derivations. Laughnan and Gabay (33) reported that several other members of the S group display similar mutability. S cytoplasm is resistant to H. maydis and Phyllosticta maydis, with possible exceptions.

Because it has only recently been positively identified, male-sterile C cytoplasm has not been intensively investigated. Beckett (5) and Duvick (15) reported that the fertility restoration of C cytoplasm is probably under sporophytic control. C cytoplasm is not easily sterilized since many inbreds possess restorer genes. The phenotype of C cytoplasm is relatively stable, at least as compared to S, and exhibits low mutability. C cytoplasm is also resistant to H. maydis and Phyllosticta maydis, with possible exceptions.

Duvick (15) reported that the potential usefulness of C cytoplasm as a replacement of T appears to be greater than that of S. The percentage of genotypes well sterilized by C is higher than that by S, and, further, a relatively high percentage of inbreds appear to be full restorers of C. Duvick (15) pointed out, however, that C cytoplasm has not yet been rigorously compared with normal cytoplasm with respect to its effect on yield and other traits of general agronomic importance, nor with respect to its stability of restoration. He concluded that much extensive performance testing should be done with C cytoplasm before it is used in producing hybrids, with much emphasis on resistance to insects and diseases.

CHAPTER III

MATERIALS AND METHODS

The basic genetic material used in the investigation presented herein was obtained from a project initiated by Josephson at the University of Tennessee in which several inbred lines were converted to various male-sterile cytoplasms (cms). The sources of cytoplasmic male sterility were crossed as female parents to several nonrestoring inbred lines. The nonrestoring inbreds were then crossed as male recurrent parents in a backcross procedure to convert each to the various male-sterile cytoplasms. Some of the converted inbreds used in the study were backcrossed only once, whereas, most of them were backcrossed at least five times. All of the male-sterile converted lines used in the test were classified as sterile according to their pollen fertility expression. The male-sterile cytoplasms, their sources, and the nonrestoring inbreds used in the study are shown in Table 1.

The converted inbreds containing the different male-sterile cytoplasms were planted in single rows in the 1975 summer breeding nursery at the Knoxville Plant Science Field Laboratory. The fertility restoring inbreds Ky21 and T115 were each crossed as male parents to two plants in each male-sterile row. Glassine bags were placed over the ear shoots of the male-sterile plants before the silks emerged to prevent their pollination by windblown pollen. The ear shoot tips were cut back one day before pollination. Several tassels in each male-parent row were bagged the day before pollen collection to prevent

Table 1. List of male-sterile cytoplasms studied, their sources, and the non-restoring inbred lines into which each cytoplasm was converted.

Sterilizable inbreds	Male-sterile cytoplasms	Cytoplasm sources
T220, T222	cms C	Charrun variety, Brazil
T232	cms G	PI 167972, Turkey
T232	cms J	Inbred line 33-16
T232	cms K	PI 177107, Turkey
T232	cms M	Inbred line M1984
T232	cms R	Reid Yellow Dent variety
T232	cms S	Stock carrying genes ij and Tp
GA209, T139, T220, T222	cms T	Golden June variety
T232	cms W	PI 172597, Turkey
T232	cms CA	PI 214199, Rainbow Flint variety
T232	cms EK	PI 221827, Early King variety
T232	cms ML	Moldavian, Russia
T232	cms MY	PI 213713, Mortgage Lifter variety
T220, T222	cms RB	Rancho Barrieto, Paraguay
T220, T222, T232	cms SC	Dr. A. Manwiller, South Carolina
T232	cms TA	Tama Flint variety
T232	cms VG	Line carrying gene Vestigial glume
GA209, T220	cms El Sal	Variety, El Salvador

contamination by foreign pollen. On pollinating days, bulk pollen, which was collected from several tassels of the male rows, was dusted over the receptive silk of the male-sterile plants. The tassel bags were then placed over the shoots to protect the developing ears.

At maturity, each crossed ear was harvested and dried. After they were individually shelled, twelve seed from each ear were packeted and sent to Homestead, Florida, where they were planted in single rows in the University of Tennessee winter nursery. Also planted in the nursery were rows of the nonrestoring inbreds with normal cytoplasm. For each converted nonrestoring inbred and male-sterile cytoplasm combination, two rows of F_1 plants representing two ears pollinated by Ky21, two rows of F_1 plants representing two ears pollinated by T115, and one row of the nonrestoring inbred with normal cytoplasm were planted in the winter nursery.

To study the inheritance of the fertility restoration characteristics of the different male-sterile cytoplasms, seed for F_2 and backcross generations were produced from each F_1 cross (nonrestoring inbred cms x restorer line). Four plants within each F_1 row were selfed to produce F_2 generation seed. The male fertility of some of the F_1 crosses was not restored in Florida, and, thus, selfing of those plants was not possible. Four additional plants in each F_1 row were backcrossed by pollen collected from the row containing the normal cytoplasm inbred. These selfed and backcrossed ears were harvested and sent back to Tennessee where they were individually shelled and packeted.

Seed from the Knoxville F_1 crosses made in 1975 along with the F_2 and backcross seed produced from the pollinations made in Florida were

planted in the 1976 summer nursery at the Knoxville Plant Science Field Laboratory. From each F_1 cross involved in the study, one F_1, two F_2, and two backcross rows were planted in each of three replications. The seed for the two F_2 rows was produced from two of the four F_1 plants selfed in Florida. The seed for the two backcross rows was produced from two of the four backcrossed plants of each F_1 row in Florida. Records were kept on each F_1 row number and the crosses made within each row in Florida to insure that the seed of the F_2 and backcross rows planted in 1976 at Knoxville were derived in pedigree origin from the same F_1 cross.

Fifty-eight F_1 crosses were included in the study. Twenty-nine of these F_1's were made using the fertility restoring inbred Ky21, while the other 29 were produced using T115 as the restorer male parent. Twenty-five seed were hand planted within each F_1, F_2, and backcross generation row. The spacing between seeds was 12 inches in rows 24 feet in length. Spacing between rows was 40 inches. Two hundred sixty-six rows comprised each replication. A list of the F_1 crosses along with the F_2 and backcross generations of each that were grown in the 1976 summer nursery at the Knoxville Plant Science Field Laboratory is shown in Tables 2 and 3.

Each of the three replications was planted on a different date to distribute the work load in note taking and also to determine the effect of planting date on the fertility restoration of the various male-sterile cytoplasms studied. The first replication was planted on April 28, 1976. A broadcast application of 710 pounds per acre of

Table 2. List of crosses using Ky21 as the restorer line, and the generations produced from these crosses that were planted in the 1976 summer nursery at the Knoxville Plant Science Field Laboratory.

Crosses	Generations
(T220 cms C x Ky21)	F_1 -- BC
(T222 cms C x Ky21)	F_1 -- BC
(T232 cms G x Ky21)	F_1 F_2 BC
(T232 cms J x Ky21)	F_1 -- BC
(T232-2 cms J x Ky21)†	F_1 F_2 BC
(T232 cms K x Ky21)	F_1 F_2 BC
(T232 cms M x Ky21)	F_1 F_2 BC
(T232 cms R x Ky21)	F_1 F_2 BC
(T232 cms S x Ky21)	F_1 F_2 BC
(GA209 cms T x Ky21)	F_1 F_2 BC
(T139 cms T x Ky21)	F_1 F_2 BC
(T220 cms T x Ky21)	F_1 F_2 BC
(T222 cms T x Ky21)	F_1 F_2 BC
(T232 cms W x Ky21)	F_1 F_2 BC
(T232 cms CA x Ky21)	F_1 F_2 BC
(T232 cms EK x Ky21)	F_1 F_2 BC
(T232 cms ML x Ky21)	F_1 F_2 BC
(T232 cms MY x Ky21)	F_1 F_2 BC
(T220 cms RB x Ky21)	F_1 -- BC
(T222 cms RB x Ky21)	F_1 -- BC
(T222-2 cms RB x Ky21)	F_1 F_2 BC
(T220 cms SC-6 x Ky21)‡	F_1 F_2 BC
(T222 cms SC-8 x Ky21)	F_1 F_2 BC
(T232 cms SC-10 x Ky21)	F_1 F_2 BC
(T232 cms TA x Ky21)	F_1 F_2 BC
(T232 cms VG x Ky21)	F_1 F_2 BC
(GA209 cms E1 Sal-11 x Ky21)	F_1 F_2 BC
(GA209 cms E1 Sal-12 x Ky21)	F_1 F_2 BC
(T220 cms E1 Sal-13 x Ky21)	F_1 -- BC

† The number (-2) following an inbred, indicates the second of the two sterile plants crossed by Ky21 in 1975 at Knoxville.

‡ The numbers succeeding cytoplasms, SC and E1 Sal, indicate different selections of the sterile cytoplasm following the original conversion cross.

Table 3. List of crosses using T115 as the restorer line, and the generations produced from these crosses that were planted in the 1976 summer nursery at the Knoxville Plant Science Field Laboratory.

Crosses	Generations
(T220 cms C x T115)	F_1 -- BC
(T222 cms C x T115)	F_1 -- BC
(T232 cms G x T115)	F_1 F_2 BC
(T232 cms J x T115)	F_1 F_2 BC
(T232 cms K x T115)	F_1 F_2 BC
(T232 cms M x T115)	F_1 F_2 BC
(T232 cms R x T115)	F_1 F_2 BC
(T232 cms S x T115)	F_1 F_2 BC
(GA209 cms T x T115)	F_1 F_2 BC
(T139 cms T x T115)	F_1 F_2 BC
(T220 cms T x T115)	F_1 F_2 BC
(T222 cms T x T115)	F_1 F_2 BC
(T232 cms W x T-15)	F_1 F_2 BC
(T232 cms CA x T115)	F_1 F_2 BC
(T232 cms EK x T115)	F_1 F_2 BC
(T232 cms ML x T115)	F_1 F_2 BC
(T232 cms MY x T115)	F_1 F_2 BC
(T220 cms RB x T115)	F_1 -- BC
(T222 cms RB x T115)	F_1 -- BC
(T220 cms SC-6 x T115)†	F_1 F_2 BC
(T222 cms SC-8 x T115)	F_1 F_2 BC
(T232 cms SC-10 x T115)	F_1 F_2 BC
(T232 cms TA x T115)	F_1 F_2 BC
(T232 cms VG x T115)	F_1 F_2 BC
(GA209 cms E1 Sal-11 x T115)	F_1 F_2 BC
(GA209-2 cms E1 Sal-11 x T115)‡	F_1 F_2 BC
(GA209 cms E1 Sal-12 x T115)	F_1 -- BC
(GA209-2 cms E1 Sal-12 x T115)	F_1 F_2 BC
(T220 cms E1 Sal-13 x T115)	F_1 -- BC

† The numbers succeeding cytoplasms, SC and E1 Sal, indicate different selections of the sterile cytoplasm following the original conversion cross.

‡ The number (-2) following an inbred, indicates the second of the two sterile plants crossed by T115 in 1975 at Knoxville.

6-12-12 fertilizer with heptachlor added was applied on April 9, with an additional 340 pounds per acre applied on April 23. Following planting, 2.0 pounds of atrazine and 2.5 pounds of alachlor per acre were applied for weed control. A sidedressing of 210 pounds per acre of ammonium nitrate was applied on June 3.

The second replication was planted on May 27. The plot area was fertilized with 1,065 pounds per acre of 6-12-12 with heptachlor added. The fertilizer was broadcast and disced in on May 26. Preemerge spraying of 2.0 pounds of atrazine and 2.5 pounds of alachlor per acre was applied following planting to control weeds. Ammonium nitrate was sidedressed at the rate of 200 pounds per acre on July 1.

The planting of the third replication was on June 8. An application of 1000 pounds per acre of 6-12-12 fertilizer with heptachlor added was broadcast and disced in on June 7. Weed control was achieved from a preemerge application of 2.0 pounds of atrazine and 2.5 pounds of alachlor per acre. A sidedressing of 200 pounds per acre of ammonium nitrate was applied on June 14.

The data collected during the summer of 1976 were based on observations of anther appearance and pollen shed of the individual F_1, F_2, and backcross generation plants during their flowering periods. At the time of flowering, plants were examined each day. Exserted anthers were classified into three groups: (1) plump, normal appearing anthers with a large pore through which pollen was shedding were classified "fertile anthers" (Random checks with a Kikken pocket microscope and iodine stain indicated that 90 to 100 percent of the

pollen grains shed by these were plump and viable in appearance.), (2) anthers which had a pore and shed some pollen, but in general looked abnormal, were classified as "partially fertile anthers" (Spot checks with the microscope indicated that 10 to 90 percent of the pollen in these anthers was viable in appearance, with the most abnormal anthers having the lowest percentage of viable-appearing pollen.), and (3) exserted anthers which had no pore and thus could not shed pollen and which were extremely shrunken were classified as "sterile anthers" (Microscopic examinations showed that these anthers virtually never contained viable-appearing pollen grains.)

Using this anther classification in conjunction with an approximate percentage of exserted anthers as a basis, each tassel was characterized into one of six classes according to a scale modified from Duvick (11) as follows:

Class I --- No anthers exserted.

Class IIA --- Fewer than half of the anthers exserted but all were sterile with no pollen shed.

Class IIB --- More than half of the anthers exserted but all were sterile with no pollen shed.

Class III --- Partially fertile anthers exserted with some pollen shed. Proportion of anthers exserted was highly variable.

Class IV --- Slightly abnormal anthers with approximately 75 to 100 percent exsertion.

Class V --- Normal anthers and fully fertile.

Data collection began on July 14, August 2, and August 9 from replications one, two, and three, respectively. The flowering stage occurred over a period of approximately 15 days in each replication. All the tassel classifications were made by the author to insure as much repeatability and consistency in making the classifications as possible.

Some of the male-sterile cytoplasms were susceptible to southern corn leaf blight and exhibited symptoms of the disease. Notes were taken on these particular rows as to the severity of the disease and its affect on the growth of the plants and their fertility expression.

The fertility data were subjected to chi-square tests of homogeneity using the IBM 360/65 computer at the University of Tennessee computing center. Frequency tables were produced from the fertility classification data of the two progeny rows of each F_2 and backcross generation. The chi-square test was used as a basis for deciding whether the progeny row data should be combined within each generation. Frequency tables were also produced from the fertility data of each generation by replication and tested by chi-square to determine any planting date effect on the fertility restoration of the various male-sterile cytoplasms.

The chi-square homogeneity tests were computed from frequency tables consisting of the six classifications of fertility ratings and also of only two classes, sterile and fertile. The sterile class was obtained by combining fertility classes I, IIA, and IIB and the fertile class by combining classes III, IV, and V.

Certain F_2 and backcross generation data also were subjected to the goodness-of-fit chi-square test to determine the similarity of genetic ratios obtained within these generations among the different male-sterile cytoplasms tested.

CHAPTER IV

RESULTS

Fertility classification data from the study as recorded in the field are on file in the office of Dr. L. M. Josephson of the Plant and Soil Science Department. Data from each F_1, F_2, and backcross generation row of the male-sterile cytoplasms crossed by the fertility restores Ky21 and T115 are included. The data are presented as the number of plants in each fertility classification for each date of planting. The combined classifications into sterile and fertile classes also are presented.

The precipitation totals and mean maximum and minimum temperatures at the Knoxville Plant Science Field Laboratory for the months May through August in 1976 are shown in Table 4. Following a cool May, there were only small temperature differences for June, July, and August. Precipitation totals appeared to be sufficient for each of the summer months; however, rainfall was less evenly distributed during August.

Temperature and rainfall data for the flowering periods of the three replications are shown in Table 5. Fifteen (5 days before to 10 days after data collection began) was arbitrarily used as the number of days for each flowering period. There was little difference in temperatures during the flowering stages of the three replications. Precipitation totals were similar for each flower period; however, the

Table 4. Mean maximum and minimum temperatures and precipitation totals at the Knoxville Plant Science Field Laboratory for months May through August in 1976.

	May	June	July	August
		Temperature, °F		
Mean maximum	73.9	84.1	85.8	85.8
Mean minimum	48.4	60.6	62.8	61.6
		Precipitation, inches		
Total	7.5	3.4	4.9	3.3
Days rained	15	9	10	6

Table 5. Mean maximum and minimum temperatures and precipitation totals for flowering periods of three replications at the Knoxville Plant Science Field Laboratory in 1976.

	Replication 1	Replication 2	Replication 3
	July 9-23	July 28 - August 11	August 4-18
		Temperature, °F	
Mean maximum	87.1	85.2	85.6
Mean minimum	62.5	61.3	60.2
		Precipitation, inches	
Total	2.1	1.9	2.2
Days rained	5	3	3

distribution of rainfall was better during the flowering stage of the first replication.

Disease infection from southern corn leaf blight occurred in genotypes containing T and SC cytoplasms. The severity of the disease increased in these genotypes as the season progressed. The disease symptoms were light in the first replication, but became moderate in the second and quite severe in the third replication. The most severe blight infections occurred in genotypes containing T139-T and GA209-T. Extreme stunting and even death of plants occurred in many instances. The fertility expression of the tassels of infected plants was inhibited.

There were 312 paired progeny rows of F_2 and backcross generations. Both two- and six-classification data from these rows were subjected to chi-square tests of homogeneity. Of the 624 progeny row comparisons tested by chi-square, only 46 were shown to be heterogeneous at the .05 level of probability. Only four progeny row comparisons were found to be heterogeneous in more than one replication within a generation of the same genotype (Table 6). Since only a small percentage of the progeny row comparisons were heterogeneous, and with only four heterogeneous comparisons being repeated in the second replication, indicates there was little or no genetic difference between progeny rows throughout the study. It also indicates that the classification system was reasonably reliable.

Chi-square tests of homogeneity showed that significant differences in fertility expression among replications occurred more in cytoplasms

Table 6. List of heterogeneous progeny rows of F_2 and backcross generations that occurred in more than one replication, as tested by chi-square.

F_1 Cross	Genera-tion	Fertility classes	Repli-cation	df	χ^2
T222-C x Ky21	BC	two	2	1	5.49*
T222-C x Ky21	BC	two	3	1	9.98**
T232-2-J x Ky21	BC	two	1	1	5.67*
T232-2-J x Ky21	BC	two	2	1	7.53**
T222-T x T115	F_2	two	1	1	5.25*
T222-T x T115	F_2	two	2	1	4.50*
GA209-E1 Sa1-12 x Ky21	F_2	six	2	3	9.20*
GA209-E1 Sa1-12 x Ky21	F_2	six	3	3	12.00**

*,** Significant at the .05 and .01 levels of probability, respectively.

C, RB, El Sal, T, and SC. Heterogeneity among replications of T and SC cytoplasms was probably due, for the most part, to the effect of southern corn leaf blight on fertility expression. Generally the second and third replications of heterogeneous generations containing T and SC cytoplasms exhibited more sterility than the first replication. This trend corresponded with the increased severity of the blight symptoms as the season progressed.

The difference in planting date apparently was responsible for the heterogeneity among replications of the other cytoplasms. Most genotypes, in which chi-square showed heterogeneity among replications, produced a larger percentage of sterile plants in the later plantings. A list of F_1 crosses in which heterogeneity among replications occurred is shown in Table 7.

The SC cytoplasm appears to belong to the T cytoplasm group. In addition to being susceptible to southern corn leaf blight, F_1, F_2, and backcross generations reacted similarly in fertility expression to T cytoplasm, when crossed to the fertility restoring inbreds Ky21 and T115. Fertility data of the inbreds containing T and SC cytoplasms crossed to the restorer lines are shown in Table 8. The F_1 plants produced by using the fertility restoring parent Ky21 were all fertile. With the exception of crosses, T232-SC-10 x T115 and T139 x T115, in which one and five plants, respectively, were recorded as sterile, T115 restored fertility completely. Most of these fertile plants, as well as the plants restored by Ky21, were classified V. The sterility of the few recorded sterile plants was probably due to the effect of blight rather than lack of fertility restoring genes.

Table 7. List of F_1 crosses in which replications were heterogeneous as tested by chi-square.

Cross	Fertility classification			
	Two class		Six class	
	df	χ^2	df	χ^2
T220-C x Ky21	2	53.01**	6	53.38**
T220-C x T115	2	11.59**	4	16.78**
T222-C x Ky21	2	10.48**	6	25.23**
T139-T x T115	2	8.99*	6	61.00**
T220-RB x Ky21	2	54.54**	8	67.00**
T220-RB x T115	2	21.01**	6	37.95**
T222-RB x Ky21	2	19.72**	6	20.59**
T222-RB x T115	2	25.21**	6	30.59**
T222-SC-8 x Ky21			4	15.24**
GA209-E1 Sal-11 x Ky21			4	55.51**
GA209-E1 Sal-11 x T115			6	19.38**
GA209-E1 Sal-12 x Ky21			4	58.11**
GA209-2-E1 Sal-12 x T115			6	20.77**
T220-E1 Sal-13 x Ky21	2	22.43**	8	28.08**

*,** Significant at the .05 and .01 levels of probability, respectively.

Table 8. The fertility restoring effect of Ky21 and T115 on SC and T cytoplasms as expressed in the F_1 populations.

Female parent	Replication	Fertility restoring parent: Ky21 Fertile	Ky21 Sterile	Replication	T115 Fertile	T115 Sterile
T220-SC-6	1,2,3†	70	0	1,2,3	71	0
T222-SC-8	1,2,3	64	0	1,2,3	72	0
T232-SC-10	1,2,3	73	0	1,2,3	71	1
GA209-T	1,2,3	69	0	1,2,3	69	0
T139-T	1,2,3	68	0	1‡	21	0
				2	17	0
				3	18	5
T220-T	1,2,3	65	0			
T222-T	1,2,3	61	0	1,2,3	64	0

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

The fertility data from F_2 generations of T cytoplasm crossed by the restoring inbreds Ky21 and T115 are shown in Table 9. The F_2 generations in which replications were homogeneous, segregated 3 fertile: 1 sterile, as tested by chi-square. This genetic ratio indicates a single dominant gene for restoration. When total plants for three replications were considered, three of the F_2 generations in which replications were heterogeneous, did not segregate in a 3:1 ratio. Data from the first replication of these F_2 generations did, however, fit a 3:1 ratio. The occurrence of southern corn leaf blight apparently biased the data of the later planted replications.

Chi-square tests for goodness-of-fit for F_2 generations of SC cytoplasm with Ky21 and T115 as restoring inbreds are shown in Table 10. The F_2 population from the cross, T232-SC-10 x Ky21, was the only one that failed to segregate 3 fertile: 1 sterile. Data from the first replication of this F_2 did, however, fit a 3:1 ratio.

Backcross generations of T cytoplasm are shown in Table 11. Most of the backcrosses segregated 1 fertile: 1 sterile, indicating a single dominant fertility restoring gene. Two of the backcrosses, of which replications were heterogeneous, did not fit a 1:1 ratio. Data from the first and third replications of ((T139-T x T115) x T139) and the first replication of ((T222-T x Ky21) x T222) did, however, segregate 1:1. Data from backcross ((T222-T x T115) x T222), of which replications were homogeneous, deviated significantly from a 1:1 ratio.

Chi-square tests for goodness-of-fit of backcross generations of SC cytoplasm were similar to backcross generation results of T cytoplasm.

Table 9. Chi-square tests for goodness-of-fit for F_2 generations of T cytoplasm.

F_1 cross	Replication	Number of plants per fertility class		χ^2 for 3:1 ratio
		Fertile	Sterile	
GA209-T x Ky21	1,2,3†	111	30	1.04
GA209-T x T115	1,2,3	99	30	0.24
T139-T x Ky21	1‡	31	8	0.42
	2	26	14	
	3	20	20	
		77	42	6.72**
T139-T x T115	1	28	8	0.15
	2	11	8	
	3	16	19	
		55	35	4.44**
T220-T x Ky21	1	39	8	
	2	36	8	
	3	27	16	
		102	32	0.09
T220-T x T115	1,2,3	109	25	2.88
T222-T x Ky21	1,2,3	89	39	2.04
T222-T x T115	1	36	8	1.09
	2	26	18	
	3	25	16	
		87	42	3.93*

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

*,** Observed genetic ratios deviated significantly from expected ratios at the .05 and .01 probability levels, respectively.

Table 10. Chi-square tests for goodness-of-fit for F_2 generations of SC cytoplasm.

F_1 cross	Replication	Number of plants per fertility class		χ^2 for 3:1 ratio
		Fertile	Sterile	
T220-SC-6 x Ky21	1,2,3†	107	28	1.30
T220-SC-6 x T115	1,2,3	104	35	0.002
T222-SC-8 x Ky21	1‡	40	5	
	2	31	15	
	3	24	20	
		95	40	1.54
T222-SC-8 x T115	1	36	4	
	2	22	14	
	3	23	20	
		81	38	3.05
T232-SC-10 x Ky21	1	42	7	3.00
	2	29	18	
	3	22	24	
		93	49	6.84**
T232-SC-10 x T115	1	33	11	
	2	24	18	
	3	38	8	
		94	37	0.74

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

** Observed genetic ratio deviated significantly from expected ratio at the .01 probability level.

Table 11. Chi-square tests for goodness-of-fit for backcross generations of T cytoplasm.

Backcross	Replication	Number of plants per fertility class		χ^2 for 1:1 ratio
		Fertile	Sterile	
(GA209-T x Ky21) x GA209	1,2,3†	72	66	0.26
(GA209-T x T115) x GA209	1‡	15	13	
	2	6	23	
	3	17	18	
		38	54	2.78
(T139-T x Ky21) x T139	1,2,3	63	76	1.22
(T139-T x T115) x T139	1	15	23	1.68
	2	2	26	20.58**
	3	19	21	0.10
		36	70	10.90**
(T220-T x Ky21) x T220	1	15	17	
	2	14	5	
	3	9	19	
		38	41	0.11
(T220-T x T115) x T220	1,2,3	64	61	0.07
(T222-T x Ky21) x T222	1	20	20	0.00
	2	7	26	10.94**
	3	15	30	5.00*
		42	72	9.79**
(T222-T x T115) x T222	1,2,3	52	92	11.11**

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

** Observed genetic ratios deviated significantly from expected ratios at the .01 level of probability.

Five of the six backcrosses segregated 1 fertile: 1 sterile as shown in Table 12. The backcross not segregating 1:1 contained the same restorer line (T115) and recurrent parent (T222) as the one not fitting a 1:1 ratio in T cytoplasm.

The 1:1 segregation of fertile to sterile plants of the backcross generations of T and SC cytoplasms further indicate the action of a single dominant gene responsible for fertility restoration. The similarities of the segregating ratios of T and SC cytoplasms in combinations with the same sterile and restorer parents, imply that they belong to the same group.

The F_1 data of cytoplasms G, J, K, M, R, S, W, CA, EK, ML, MY, TA, and VG are shown in Table 13. These cytoplasms have previously been characterized as members of the S group (4,26,27). Since each of these cytoplasms was converted to the same inbred (T232), the data from the generations produced containing these cytoplasms are presented together and referred to collectively as the S group. Except for W cytoplasm, in which five plants were recorded as sterile, Ky21 and T115 restored pollen fertility in all cytoplasms. Most of the plants were fully restored.

The fertility restoration of S cytoplasm has been found to be under gametophytic control (8); with only those microspores containing the dominant gene for restoration, developing into viable pollen grains. Plants of F_2 generations produced from heterozygous F_1 plants would therefore be expected to produce only fertile tassels. The F_2 data shown in Tables 14 and 15 indicate that some sterile plants were

Table 12. Chi-square tests for goodness-of-fit for backcross generations of SC cytoplasm.†

Backcross	Number of plants per fertility class		χ^2 for 1:1 ratio
	Fertile	Sterile	
(T220-SC-6 x Ky21) x T220	64	62	0.03
(T220-SC-6 x T115) x T220	68	73	0.17
(T222-SC-8 x Ky21) x T222	52	64	1.24
(T222-SC-8 x T115) x T222	38	92	22.43**
(T232-SC-10 x Ky21) x T232	63	67	0.12
(T232-SC-10 x T115) x T232	68	76	0.44

† Replications of all backcross generations were homogeneous as tested by chi-square (.05 probability). Data presented as total plants of three replications.

** Observed genetic ratio deviated significantly from expected ratio at the .01 probability level.

Table 13. The fertility restoring effect of Ky21 and T115 on several S-group cytoplasms as expressed in the F_1 populations.†

Inbred and cytoplasm	Fertility restoring parent			
	Ky21		T115	
	Fertile	Sterile	Fertile	Sterile
T232-G	69	0	75	0
T232-J	66	0		
T232-2-J	74	0	63	0
T232-K	72	0	74	0
T232-M	73	0	72	0
T232-R	72	0	47	0
T232-S	73	0	51	0
T232-W	69	5	69	0
T232-CA	65	0	74	0
T232-EK	65	0	72	0
T232-ML	69	0	74	0
T232-MY	69	0	71	0
T232-TA	71	0	70	0
T232-VG	71	0	74	0

† Replications of all F_1 populations were homogeneous as tested by chi-square (.05 probability). Data presented as total plants of three replications.

Table 14. Chi-square tests for goodness-of-fit of F_2 generations of several S-group cytoplasms with Ky21 as the restorer line.

F_1 cross	Replication	Number of plants per fertility class		χ^2 for 15:1 ratio
		Fertile	Sterile	
T232-G x Ky21	1,2,3†	121	11	0.97
T232-2-J x Ky21	1,2,3	133	10	0.13
T232-K x Ky21	1,2,3	134	10	0.12
T232-M x Ky21	1,2,3	102	12	3.56
T232-R x Ky21	1,2,3	127	14	3.26
T232-S x Ky21	1‡	35	8	11.20**
	2	45	3	0.00
	3	47	2	0.39
		127	13	2.20
T232-W x Ky21	1,2,3	135	10	0.10
T232-CA x Ky21	1,2,3	132	12	1.06
T232-EK x Ky21	1,2,3	115	15	6.20*
T232-ML x Ky21	1,2,3	113	21	20.30**
T232-MY x Ky21	1,2,3	130	12	1.17
T232-TA x Ky21	1,2,3	133	12	3.01
T232-VG x Ky21	1,2,3	117	14	4.40*

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

*,** Observed genetic ratios deviated significantly from expected ratios at the .05 and .01 probability levels, respectively.

Table 15. Chi-square tests for goodness-of-fit of F_2 generations of several S-group cytoplasms with T115 as the restorer line.

F_1 cross	Replication	Number of plants per fertility class		χ^2 for 15:1 ratio
		Fertile	Sterile	
T232-G x T115	1,2,3†	137	4	2.80
T232-2-J x T115	1,2,3	130	6	0.78
T232-K x T115	1,2,3	132	9	0.01
T232-M x T115	1,2,3	108	6	0.18
T232-R x T115	1,2,3	113	16	5.12*
T232-S x T115	1,2,3	139	4	2.90
T232-W x T115	1,2,3	139	8	0.16
T232-CA x T115	1,2,3	131	11	0.54
T232-EK x T115	1,2,3	140	5	1.95
T232-ML x T115	1,2,3	103	13	4.86*
T232-MY x T115	1‡	46	0	3.06
	2	39	7	6.31*
	3	50	0	3.33
		135	7	0.42
T232-TA x T115	1,2,3	123	16	6.56*
T232-VG x T115	1,2,3	137	7	0.47

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

* Observed genetic ratios deviated significantly from expected ratios at the .05 probability level.

produced. Duvick (14) reported that in some genotypes, a small percentage of fertile pollen grains containing the recessive restoring gene are produced by heterozygous plants. Chi-square tests for goodness-of-fit show that a majority of the F_2 populations with both Ky21 and T115 used as restorer lines, segregated 15 fertile: 1 sterile. These results are in agreement with F_2 data obtained from J cytoplasm by Josephson and Kincer.[1] If the fertility restoration is under sporophytic control, the 15:1 ratio suggests that duplicate dominant epistasis is responsible for fertility restoration.

All backcrosses in the study were made using F_1 crosses containing the male-sterile cytoplasm as seed parents. In S-group cytoplasms, this would eliminate the gametophytic factor from upsetting the segregation ratios of backcrosses. Segregation is normal for a single gene when heterozygous plants containing S-type cytoplasms are used as female parents in crosses (8). Most of the backcrosses of the S-group cytoplasms with T115 as the restorer line segregated 1 fertile: 1 sterile, as shown in Table 16. This segregation ratio indicates that a single dominant gene is responsible for fertility restoration of these cytoplasms. S cytoplasm, however, along with cytoplasms ML and TA deviated from the 1:1 backcross ratio.

Backcrosses containing the S-group cytoplasms with Ky21 as the restorer line, produced different segregation patterns as shown in

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[1]Josephson, L. M. and H. C. Kincer, (Annual unpublished Report of Corn Breeding Project, Univ. of Tennessee, 1965).

Table 16. Chi-square tests for goodness-of-fit for backcross generations of several S-group cytoplasms with T115 as the restorer line.†

Backcross	Number of plants per fertility class		χ^2 for 1:1 ratio
	Fertile	Sterile	
(T232-G x T115) x T232	80	65	1.55
(T232-2-J x T115) x T232	81	59	3.46
(T232-K x T115) x T232	63	70	0.37
(T232-M x T115) x T232	78	61	2.08
(T232-R x T115) x T232	73	69	0.11
(T232-S x T115) x T232	91	48	13.30**
(T232-W x T115) x T232	80	63	2.02
(T232-CA x T115) x T232	70	66	0.12
(T232-EK x T115) x T232	78	59	2.63
(T232-ML x T115) x T232	91	51	11.26**
(T232-MY x T115) x T232	76	59	2.14
(T232-TA x T115) x T232	82	58	4.11*
(T232-VG x T115 x T232	68	65	0.07

† Replications of all backcross generations were homogeneous as tested by chi-square (.05 probability). Data presented as total plants of three replications.

*,** Observed genetic ratios deviated significantly from expected ratios at the .05 and .01 levels of probability, respectively.

Table 17. The backcross containing R cytoplasm was the only cross, of which replications were homogeneous, that segregated 1 fertile: 1 sterile. Data from the second replications for each of the three backcrosses, of which replications were heterogeneous, segregated 1:1. Also listed in Table 17 are chi-square tests for goodness-of-fit for backcross ratios of 3 fertile: 1 sterile. Data from four of the 11 backcrosses, of which replications were homogeneous, fit a 3:1 ratio. Total plants in two of the three backcrosses, of which replications were heterogeneous, segregated 3:1. The first and third replications of backcross ((T232-J x Ky21) x T232), of which replications were heterogeneous, segregated 3:1.

Date of planting had a considerable effect on fertility restoration of C cytoplasm. The F_1 crosses grown in Florida were sterile, thus preventing the production of F_2 seed. The F_1 and backcross generations of C cytoplasm with Ky21 as the restorer line are shown in Table 18. The fertility of most of the F_1 plants of the sterile inbred T220 was restored in the first replication. A majority of the plants classified fertile were of the partially fertile classes III and IV. However, all but one plant of the second and third planting dates were sterile. The fertility of T222-C x Ky21 was restored in the first replication. These fertile plants were all partially fertile. However, a large percentage of plants in the second and third replications were sterile. The backcross generations produced a majority of sterile plants. The few plants classified as fertile were partially fertile.

The fertility restoration of C cytoplasm was even less with inbred T115, as shown in Table 19. Only a few fertile plants were produced in

Table 17. Chi-square tests for goodness-of-fit for backcross generations of several S-group cytoplasms with Ky21 as the restorer line.

Backcross	Replication	Number of plants per fertility class		χ^2 for ratios	
		Fertile	Sterile	1:1	3:1
(T232-G x Ky21) x T232	1,2,3†	94	46	16.46**	4.60*
(T232-J x Ky21) x T232	1‡	36	12	12.00**	0.00
	2	24	26	0.08	19.44**
	3	33	15	6.75**	1.00
		93	53	10.95**	9.94**
(T232-2-J x Ky21) x T232	1,2,3	99	45	20.25**	3.00
(T232-K x Ky21) x T232	1,2,3	98	47	17.93**	4.25*
(T232-M x Ky21) x T232	1,2,3	104	38	30.67**	0.23
(T232-R x Ky21) x T232	1,2,3	80	67	1.14	33.13**
(T232-S x Ky21) x T232	1,2,3	105	36	33.76**	0.02
(T232-W x Ky21) x T232	1,2,3	90	50	11.42**	8.57**
(T232-CA x Ky21) x T232	1,2,3	89	54	8.56**	12.42**
(T232-EK x Ky21) x T232	1,2,3	86	53	7.83**	12.77**
(T232-ML x Ky21) x T232	1,2,3	85	59	4.69*	19.59**
(T232-MY x Ky21) x T232	1	35	11	12.52**	0.03
	2	20	20	0.00	13.33**
	3	37	12	12.76**	0.007
		92	43	17.78**	3.38
(T232-TA x Ky21) x T232	1,2,3	109	27	49.44**	1.92
(T232-VG x Ky21) x T232	1	34	13	9.38**	0.18
	2	30	19	2.46	4.96*
	3	42	5	29.13**	5.17*
		106	37	33.29**	0.05

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

*,** Observed genetic ratios deviated significantly from expected ratios at the .05 and .01 levels of probability, respectively.

Table 18. Fertility data for F_1 and backcross generations of C cytoplasm with Ky21 as the restorer parent.

Pedigree	Generation	Replication	Number of plants per fertility class	
			Fertile	Sterile
T220-C x Ky21	F_1	1†	20	3
		2	0	22
		3	1	24
(T220-C x Ky21) x T220	BC	1	11	33
		2	0	49
		3	0	46
T222-C x Ky21	F_1	1	21	0
		2	11	7
		3	16	9
(T222-C x Ky21) x T222	BC	1,2,3‡	23	120

† Heterogeneous replications as tested by chi-square (.05 probability).

‡ Homogeneous replications as tested by chi-square (.05 probability).

Table 19. Fertility data for F_1 and backcross generations of C cytoplasm with T115 as the restorer parent.

Pedigree	Generation	Replication	Number of plants per fertility class	
			Fertile	Sterile
T220-C x T115	F_1	1†	5	16
		2	0	22
		3	0	23
(T220-C x T115) x T220	BC	1,2,3‡	6	142
T222-C x T115	F_1	1,2,3	0	58
(T222-C x T115) x T222	BC	1,2,3	1	137

† Heterogeneous replications as tested by chi-square (.05 probability).

‡ Homogeneous replications as tested by chi-square (.05 probability).

either F_1 or backcross generations. These plants, however, were only partially restored. The restoration of C cytoplasm was too variable to test any segregating ratios by chi-square tests for goodness-of-fit.

The fertility expression of RB cytoplasm was similar to that of C. The fertility restoration pattern of F_1 and backcross generations of T220-RB crossed by Ky21, as shown in Table 20, was nearly identical to that of C cytoplasm with the same sterile inbred and restorer line. The few fertile plants were produced only in the first replication, and most of these were only partially restored. The sterile inbred, T222-RB, varied in its fertility restoration response when crossed by Ky21. One of the two F_1 ears (T222-2-RB x Ky21) made at Knoxville in 1975 was fertile in Florida. When grown at Knoxville in 1976, as shown in Table 20, most of the plants of this F_1 were partially restored. The data for the F_2 and backcross generations produced from this F_1 fit segregating ratios of 9 fertile: 7 sterile and 1 fertile: 3 sterile, respectively, indicating two dominant genes necessary for fertility restoration. The data are too variable, however, to make such an assumption with any degree of confidence.

The restoration of RB cytoplasm by T115 was very similar to the fertility restoration of the same sterile inbreds containing C cytoplasm. As shown in Table 21, T115 did not fully restore the fertility of RB cytoplasm in sterile inbreds T220 and T222. Most of the replications were heterogeneous, indicating environmental influences on the fertility restoration of RB cytoplasm.

The fertility restoration data of El Sal cytoplasm by Ky21 are shown in Table 22. Fertility of the two F_1 populations of sterile

Table 20. Fertility data for F_1, F_2, and backcross generations of RB cytoplasm with Ky21 as the restorer parent.

Pedigree	Generation	Replication	Number of plants per fertility class	
			Fertile	Sterile
T220-RB x Ky21	F_1	1†	20	3
		2	0	22
		3	0	22
(T220-RB x Ky21) x T220	BC	1	12	36
		2	0	46
		3	0	48
			12	130
T222-RB x Ky21	F_1	1	8	15
		2	22	3
		3	21	4
(T222-RB x Ky21) x T222	BC	1	16	29
		2	8	39
		3	19	26
			43	94
T222-2-RB x Ky21	F_1	1,2,3‡	62	7
T222-2-RB x Ky21	F_2	1,2,3	81	57
(T222-2-RB x Ky21) x T222	BC	1	16	34
		2	6	42
		3	18	29
			40	105

† Heterogeneous replications as tested by chi-square (.05 probability).

‡ Homogeneous replications as tested by chi-square (.05 probability).

Table 21. Fertility data for F_1 and backcross generations of RB cytoplasm with T115 as the restorer parent.

Pedigree	Genera-tion	Repli-cation	Number of plants per fertility class	
			Fertile	Sterile
T220-RB x T115	F_1	1†	8	5
		2	0	15
		3	1	18
(T220-RB x T115) x T220	BC	1,2,3‡	0	143
T222-RB x T115	F_1	1	2	22
		2	0	22
		3	14	11
(T222-RB x T115) x T222	BC	1	8	42
		2	0	49
		3	1	48

† Heterogeneous replications as tested by chi-square (.05 probability).

‡ Homogeneous replications as tested by chi-square (.05 probability).

Table 22. Fertility data for F_1, F_2, and backcross generations of El Sal cytoplasm with Ky21 as the restorer parent.

Pedigree	Genera-tion	Repli-cation	Number of plants per fertility class	
			Fertile	Sterile
GA209-El Sal-11 x Ky21	F_1	1,2,3†	73	0
GA209-El Sal-11 x Ky21	F_2	1‡	31	18
		2	22	26
		3	34	15
			87	59
(GA209-El Sal-11 x Ky21) x GA209	BC	1,2,3	58	85
GA209-El Sal-12 x Ky21	F_1	1,2,3	73	0
GA209-El Sal-12 x Ky21	F_2	1,2,3	72	55
(GA209-El Sal-12 x Ky21) x GA209	BC	1,2,3	69	78
T220-El Sal-13 x Ky21	F_1	1	18	6
		2	4	18
		3	4	20
(T220-El Sal-13 x Ky21) x T220	BC	1	14	30
		2	1	44
		3	0	49
			15	123

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

inbred GA209 was restored by Ky21. The plants of the first replication of both were fully fertile. Partially fertile plants comprised much of the second and third replications. The F_2 generations of these two F_1 populations segregated 9 fertile: 7 sterile; however, the backcrosses failed to segregate according to a two dominant gene hypothesis. The F_1 and backcross generations of sterile inbred T220 containing El Sal cytoplasm reacted similarly in fertility expression to C and RB cytoplasms when crossed to Ky21, as shown in Table 23.

The fertility of inbred GA209 with El Sal cytoplasm was not restored as much by T115 as with Ky21, as shown in Table 24. The F_2 and backcross generation data were too variable to test any hypotheses regarding number of restorer genes. The fertility restoration effect of T115 on sterile inbred T220 with El Sal cytoplasm was similar to that of C and RB cytoplasms, as shown in Table 25.

Table 23. Fertility data for F_1 and backcross generations of C, RB, and E1 Sa1 cytoplasms with T220 as the sterile parent and Ky21 as the restorer line.

Pedigree	Genera-tion	Repli-cation	Number of plants per fertility class	
			Fertile	Sterile
T220-C x Ky21	F_1	1†	20	3
		2	0	22
		3	1	24
T220-RB x Ky21	F_1	1	20	3
		2	0	22
		3	0	22
T220-E1 Sa1-13 x Ky21	F_1	1	18	6
		2	4	18
		3	4	20
(T220-C x Ky21) x T220	BC	1	11	33
		2	0	49
		3	0	46
(T220-RB x Ky21) x T220	BC	1	12	36
		2	0	46
		3	0	48
(T220-E1 Sa1-13 x Ky21) x T220	BC	1	14	30
		2	1	44
		3	0	49

† Heterogeneous replications as tested by chi-square (.05 probability).

Table 24. Fertility data for F_1, F_2, and backcross generations of E1 Sal cytoplasm with T115 as the restorer parent.

Pedigree	Generation	Replication	Number of plants per fertility class	
			Fertile	Sterile
GA209-E1 Sal-11 x T115	F_1	1,2,3†	67	3
GA209-E1 Sal-11 x T115	F_2	1‡	29	17
		2	13	34
		3	10	35
			52	86
(GA209-E1 Sal-11 x T115) x GA209	BC	1	26	23
		2	6	43
		3	18	27
			50	93
GA209-2-E1 Sal-11 x T115	F_1	1,2,3	59	13
GA209-2-E1 Sal-11 x T115	F_2	1	31	17
		2	16	31
		3	24	22
			71	70
(GA209-2-E1 Sal-11 x T115) x GA209	BC	1,2,3	40	93
GA209-E1 Sal-12 x T115	F_1	1,2,3	67	5
(GA209-E1 Sal-12 x T115) x GA209	BC	1	26	23
		2	14	34
		3	15	31
			55	88
GA209-2-E1 Sal-12 x T115	F_1	1,2,3	59	8
GA209-2-E1 Sal-12 x T115	F_2	1	27	6
		2	13	17
		3	26	11
			66	34
(GA209-2-E1 Sal-12 x T115) x GA209	BC	1	27	23
		2	9	35
		3	23	26
			59	84

Table 24. (continued)

Pedigree	Genera-tion	Repli-cation	Number of plants per fertility class	
			Fertile	Sterile
T220-E1 Sal-13 x T115	F_1	1,2,3	3	63
(T220-E1 Sal-13 x T115) x T220	BC	1,2,3	1	141

† Homogeneous replications as tested by chi-square (.05 probability).

‡ Heterogeneous replications as tested by chi-square (.05 probability).

Table 25. Fertility data for F_1 and backcross generations of C, RB, and E1 Sal cytoplasms with T220 as the sterile parent and T115 as the restorer line.

Pedigree	Genera-tion	Repli-cation	Number of plants per fertility class	
			Fertile	Sterile
T220-C x T115	F_1	1†	5	16
		2	0	22
		3	0	23
T220-RB x T115	F_1	1	8	5
		2	0	15
		3	1	18
T220-E1 Sal-13 x T115	F_1	1,2,3‡	3	63
(T220-C x T115) x T220	BC	1,2,3	6	142
(T220-RB x T115) x T220	BC	1,2,3	0	143
(T220-E1 Sal-13 x T115) x T220	BC	1,2,3	1	141

† Heterogeneous replications as tested by chi-square (.05 probability).

‡ Homogeneous replications as tested by chi-square (.05 probability).

CHAPTER V

DISCUSSION

Fertility of the sterile inbreds containing T cytoplasm was fully restored by Ky21. T115 also fully restored the fertility of the sterile inbreds containing T cytoplasm except for five plants in the third replication of the cross T139-T x T115. A majority of the F_1 plants restored by Ky21 and T115 were classified V.

The fertility of sterile inbreds containing SC cytoplasm also was fully restored when crossed by Ky21. Inbred T115 restored fertility in all F_1 plants containing SC cytoplasm except for one plant in the cross T232-SC-10 x T115. Most of the fertile F_1 plants restored by Ky21 and T115 were classified V.

From previous studies based on their reaction in certain inbreds, cytoplasms G, J, K, M, R, S, W, CA, EK, ML, MY, TA, and VG have been characterized as belonging to the S group of male-sterile cytoplasms. The fertility of inbred T232 containing each of the male-sterile cytoplasms was restored by Ky21 in every cross except for T232-W x Ky21, of which five plants were sterile. Male fertility was restored in all F_1 plants of T232 containing the S-group cytoplasms when crossed by T115. The fertility classification of most restored F_1 plants crossed by Ky21 and T115 was V, or fully fertile.

It is apparent from the fertility expression of these F_1 plants that inbreds Ky21 and T115 are full restorers of fertility for T, SC, and the S-group male-sterile cytoplasms.

The fertility of inbreds T220 and T222 containing C, RB, and E1 Sal cytoplasms was partially restored by Ky21 and T115 at Knoxville. Most of the fertile F_1 plants were classified III and IV. Inbred Ky21 restored fertility in more plants than T115. GA209 containing E1 Sal cytoplasm was restored more than inbred T220 with this cytoplasm. The fertility in all F_1 plants in two crosses of GA209-E1 Sal crossed by Ky21 was restored. Most of these plants, however, were only partially fertile.

Inbreds Ky21 and T115 appear to be only partial restorers of cytoplasms C, RB, and E1 Sal, although Ky21 restored fertility of more F_1 plants than did T115.

The data from F_2 and backcross generations containing T cytoplasm indicated a single dominant gene necessary to restore fertility of the sterile inbreds studied. This is in agreement with previous studies which have shown that most inbreds contain the homozygous dominant allele, Rf_2; whereas, the second major allele (Rf_1) necessary for fertility restoration is generally absent (9,14,16,25). Apparently inbreds GA209, T139, T220, and T222 contain Rf_2 in the homozygous dominant form. Replications were heterogeneous in certain generations, with total number of plants deviating significantly from the expected ratios associated with a single dominant gene hypothesis. The ratios obtained from plants of the first replications of these generations did, however, indicate the action of a single dominant restoring gene. The occurrence of southern corn leaf blight apparently inhibited the fertility expression of the plants in the late plantings.

The inheritance of fertility restoration of SC cytoplasm was similar to that of T. The genetic ratios obtained from F_2 and backcross generations containing SC cytoplasm indicate a single dominant gene necessary for fertility restoration. Only one of the six backcross generations exhibited a genetic ratio that deviated significantly from a single-dominant-gene hypothesis. This cross contained T222 as the sterile inbred and T115 as the restoring inbred, both of which are normally poor pollen producers.

The use of poor pollen producers in a study of this nature could bias the results. Some genotypes tend to have appreciable numbers of abnormal anthers, even in normal cytoplasm. This is especially true for some inbred lines, with certain unfavorable seasons bringing out the trait quite strongly. The abnormal anthers are similar in appearance to anthers which are partially fertile or sterile due to the action of male-sterile cytoplasms. Therefore, all segregating populations should be grown in both male-sterile and normal cytoplasms in order to be sure one is not confusing two separate types of inheritance.

The variation in fertility expression among F_2 generations containing S-group cytoplasms was minimal and should perhaps be ignored since it is assumed that pollen viability of F_2 plants in S cytoplasm is under gametophytic control. Most of the backcross generations with T115 as the restorer source segregated 1 fertile: 1 sterile, indicating a single dominant gene necessary for fertility restoration. Cytoplasms S, ML, and TA deviated significantly from a 1:1 ratio with more fertile

plants being produced than expected. The backcross generations with Ky21 as the restorer line produced considerably more fertile plants than the T115 backcrosses. The segregation pattern of only one of the Ky21 backcrosses indicated a 1:1 ratio. Several of the backcross generations produced such high numbers of fertile plants that ratios of 3 fertile: 1 sterile were obtained, indicating a two dominant-duplicate gene system of fertility restoration.

Inbred T232 was used as the sterile inbred and recurrent parent in all the S-group cytoplasm backcrosses. The difference in segregating patterns of the backcrosses with Ky21 compared to those with T115 was apparently due to genetic differences between the two restorers. Throughout the study, Ky21 consistently restored fertility more than did T115. Inbred Ky21 apparently contains more modifier alleles which tend to collectively produce a duplicate major gene effect, or approach it, in certain S-group cytoplasms.

The F_1 plants of C sterile inbreds T220 and T222 crossed by Ky21 and T115 were sterile in Florida and, thus, prevented the production of F_2 progenies. Backcrosses were made and grown in the 1976 Knoxville test. Most of the plants were classified as sterile. Inbred Ky21 restored fertility of more plants than did T115. There were not enough plants restored to test any hypothesis regarding the inheritance of fertility restoration of C cytoplasm.

Most of the F_1 crosses with RB cytoplasm were sterile in Florida. The backcrosses made from these F_1 plants reacted in a manner very similar to C cytoplasm in their fertility expression. More fertile

plants were produced from backcrosses with Ky21 as the restorer line. The F_2 and backcross generations produced from the cross, T222-2-RB x Ky21, which was fertile in Florida, segregated into ratios indicating two dominant genes necessary for fertility restoration. No definite conclusions can be drawn, however, from these limited results.

Inbred T220 in E1 Sa1 cytoplasm crossed by Ky21 and T115 was sterile in Florida. The fertility expression of the backcrosses grown at Knoxville was similar to backcrosses of T220 in C and RB cytoplasms. The fertility of GA209 in E1 Sa1 cytoplasm crossed by Ky21 and T115 was restored in Florida. The data of F_2 and backcross generations grown at Knoxville were too variable to determine the number of genes necessary for fertility restoration. The fertility of F_2 and backcross generations of GA209 in E1 Sa1 cytoplasm was restored more by Ky21, but not sufficiently to permit testing a definite hypothesis concerning restoration.

Most of the fertile plants in generations of C, RB, and E1 Sa1 cytoplasms crossed by Ky21 and T115 were classified III and IV. Apparently Ky21 and T115 do not contain the proper complement of alleles of the major or modifier gene or genes necessary to fully restore the fertility of these cytoplasms. Thus, the data from crosses involving T220, T222 and GA209 were not adequate to determine the inheritance of fertility restoration.

SC cytoplasm has not been previously characterized as to which group of male-sterile cytoplasm it belongs. Due to the similarities in fertility restoration and both being susceptible to southern corn leaf blight, T and SC appear to belong to the same cytoplasm group.

The fertility expression of cytoplasms G, J, K, M, R, S, W, CA, EK, ML, MY, TA, and VG in F_1 generations confirm their previous classification as belonging to the S-group. The cytoplasms also reacted similarly in F_2 generations. In backcross generations, however, there was variability among these male-sterile cytoplasms. Variability was observed more in backcrosses with Ky21 as the restorer. Gracen (26) observed variations within the fertility restoration reactions of certain cytoplasms previously classified in the S group. Since only one sterilized inbred and two restorers were used in this study, subgrouping could not be clearly defined from the data.

Previous classification of C and RB cytoplasms as belonging to the same group was confirmed from the data of this study. Most F_1 generations of both cytoplasms were sterile in Florida. The F_1 and backcross generation data obtained at Knoxville were similar for both C and RB cytoplasms.

Fertility expression of T220 in E1 Sa1 cytoplasm crossed by Ky21 and T115 was almost identical to that with C and RB cytoplasms. This would indicate that E1 Sa1 cytoplasm belongs to the same group as C and RB. However, the fertility expression of GA209 in E1 Sa1 cytoplasm crossed by Ky21 and T115 was different from that of inbred T220. E1 Sa1 cytoplasm appears, however, to belong to the C group.

Date of planting had little effect on fertility expression of the S-group cytoplasms. The heterogeneous replications that occurred in cytoplasms T and SC probably resulted from the effect of southern corn leaf blight. Date of planting had a considerable effect on the fertility

expression of cytoplasms C, RB, and El Sal. Apparently these male-sterile cytoplasms require more modifier genes for full restoration of fertility. Fertility was generally restored more in the first replication. Since the temperatures and precipitation totals were similar during the flowering periods of each replication, the cool temperatures of May were probably responsible for the higher number of fertile plants produced in the first replication. The fact that most of the F_1 plants of both C and RB cytoplasms were sterile in Florida, with several being partially fertile in Knoxville, is unexplainable since, in general, restored male-sterile cytoplasms tend to be more fertile in Florida.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Pollen fertility of most sterilized lines containing T, SC, and S-group male-sterile cytoplasms was fully restored by Ky21 and T115. The few sterile plants produced from the F_1 generations involving these cytoplasms were probably the result of outcrosses. Fertility of sterilized lines containing C, RB, and E1 Sa1 cytoplasms was only partially restored by Ky21 and T115. Ky21 restored the fertility in more F_1 plants containing these cytoplasms than did T115.

Backcross and F_2 generation data indicated a single gene was responsible for fertility restoration of sterilized lines containing T cytoplasm. Apparently the sterilized lines used in this study contained one of the two major genes necessary for fertility restoration of T cytoplasm. A single dominant gene also was found to be necessary for fertility restoration of sterilized lines containing SC male-sterile cytoplasm.

Most backcross generations of S-group male-sterile cytoplasms, with T115 as the restorer source, indicated a single dominant gene necessary for fertility restoration. Backcross generations with Ky21 as the restorer line produced considerably more fertile plants than did T115 backcrosses. Only one Ky21 backcross segregated according to a single-dominant-gene hypothesis. Apparently the genotypes of Ky21 and T115 differ with respect to genes associated with fertility restoration.

Most fertile plants produced from generations containing C, RB, and E1 Sa1 cytoplasms were only partially restored. The fertility data observed from crosses involving restorers Ky21 and T115 with T220, T222, and GA209 as sterilized lines were not adequate to determine the inheritance of fertility restoration of these male-sterile cytoplasms.

Cytoplasms T and SC appear to belong to the same group. Both were susceptible to southern corn leaf blight, and their fertility restoration patterns were very similar.

The fertility expression of F_1 generations containing male-sterile cytoplasms G, J, K, M, R, S, W, CA, EK, ML, MY, TA, and VG confirm their previous classification as belonging to the S-group. Some variation in fertility restoration occurred among these cytoplasms as observed from backcross generation data.

Previous classification of C and RB cytoplasms as belonging to the same group was confirmed from data obtained from crosses involving them.

Fertility expression of T220 in E1 Sa1 male-sterile cytoplasm crossed by Ky21 and T115 was very similar to that of crosses using the same restorers and sterilized line with C and RB cytoplasms. E1 Sa1 cytoplasm appears to belong to the C group.

Date of planting had little effect on fertility restoration of S-group cytoplasms. Heterogeneity among dates of planting of T and SC cytoplasms appeared to be due to the effect of southern corn leaf blight on fertility restoration of plants containing C, RB, and E1 Sa1 cytoplasms, with a higher rate of sterility appearing in the later plantings.

The results of this study on pollen restoration indicate that two restorers may differ in genotype with respect to restoration, as observed when restorers KY21 and T115 were used on the same sterile inbred. Also, different inbreds with the same source of sterile cytoplasm react differently when crossed by the same restorer. Any statement, therefore, concerning the number of genes responsible for fertility restoration of a given restorer is true only with respect to the sterilized line used as a tester. Different sterilized lines may not be recessive for the same number of fertility restoring factors. Inbred WF9 is homozygous recessive for both genes necessary for fertility restoration of T cytoplasm; whereas, most other sterilized inbreds with T cytoplasm are homozygous recessive for only one of the major genes. Thus, two investigators, each using a different sterilized inbred as a tester, could study the genetics of the same restorer line and come to opposing conclusions as to the number and action of restoring factors involved.

These observations also indicate the influence of growing conditions on the degree of fertility restoration. Depending upon the area of the country in which the study is conducted, as observed in differences between Florida and Knoxville F_1 generation data, the results obtained from two investigators could be conflicting. Differences in growing conditions throughout the season at a given location, as observed from the effect of planting date at Knoxville, also could influence an investigator's conclusions.

Thus, inheritance studies of fertility restoration should be conducted using several sterile and restorer line combinations for each male-sterile cytoplasm studied. These studies should be conducted over a wide range of growing conditions in order to reveal the presence of any environmental influence.

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