CHAPTER SIXTEEN

Genetic Male Sterility for Population Improvement

Genetic male sterility can be used to facilitate cross-pollination in species with a high degree of natural self-pollination. The genetic control of male sterility generally is by single recessive nuclear alleles, although more complex genetic systems have been reported. In this section, a single recessive allele (*ms*) will be used to illustrate the procedures that can be employed with genetic male sterility. For the procedures to be used for other systems of genetic control, appropriate segregation patterns for sterility would have to be determined.

The primary use of genetic male sterility in breeding programs is to facilitate population improvement by recurrent selection. Alternative methods can be employed to improve a population into which male sterility has been incorporated. Selection within the populations can be based on individual plants, progeny evaluation, or both.

DEVELOPMENT OF A POPULATION

Populations to be used for recurrent selection are developed with male-fertile parents that possess the desired characteristics. A source of the *ms* allele for male sterility is obtained from a parent with as many desirable characteristics as possible.

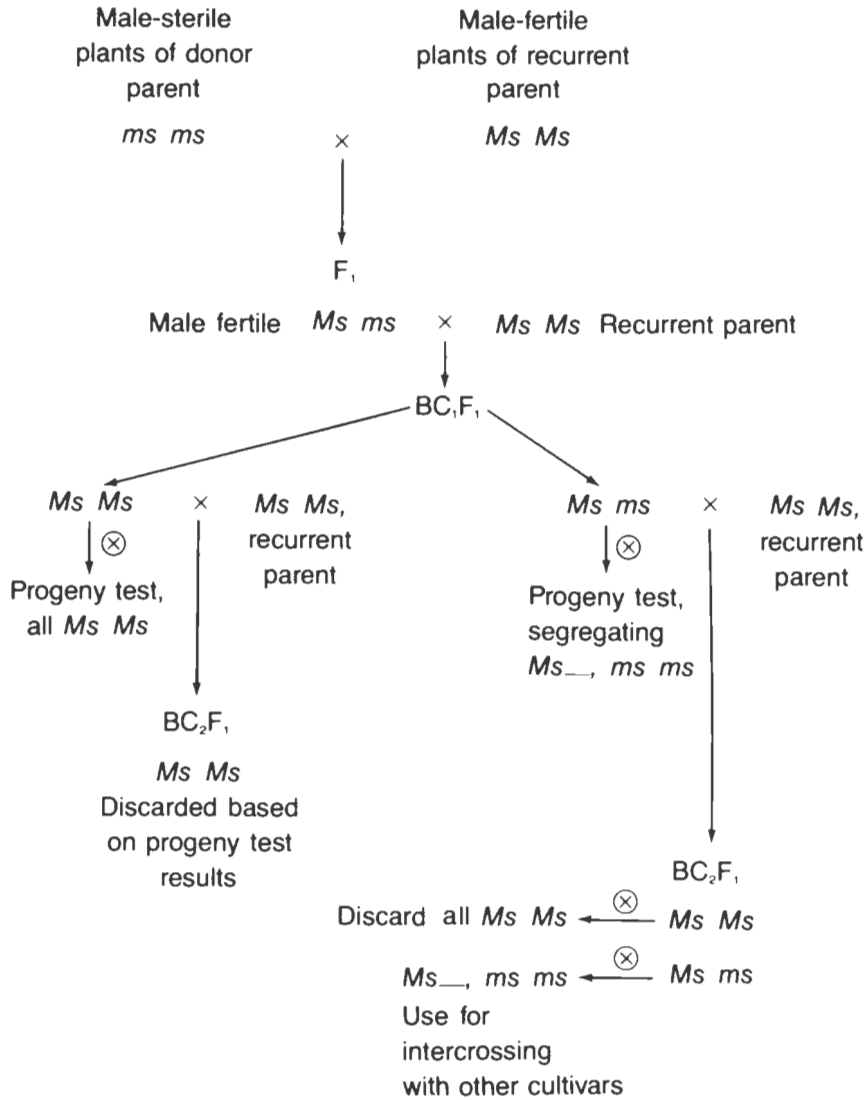
One consideration in developing the population is the percentage of genes from the male-sterile parent, other than the male-sterile (*ms*) allele, that is preferred by the breeder. A percentage less than 50 percent requires backcrossing to the male-fertile parents.

A second consideration in population development is the number of generations of recombination that are to be accomplished before selection begins.

Recombination is easily accomplished after the male-sterility gene has been incorporated into the population, but each generation of recombination requires additional time.

A procedure for population development is illustrated in the following description and in Fig. 16-1 and 16-2. It is assumed that the genetic contribution

Figure 16-1 Incorporation of a recessive allele (*ms*) for genetic male sterility into a recurrent parent. The assumption for illustration purposes is that the desired level of alleles from the recurrent parent is 87.5 percent, attained after two backcrosses.



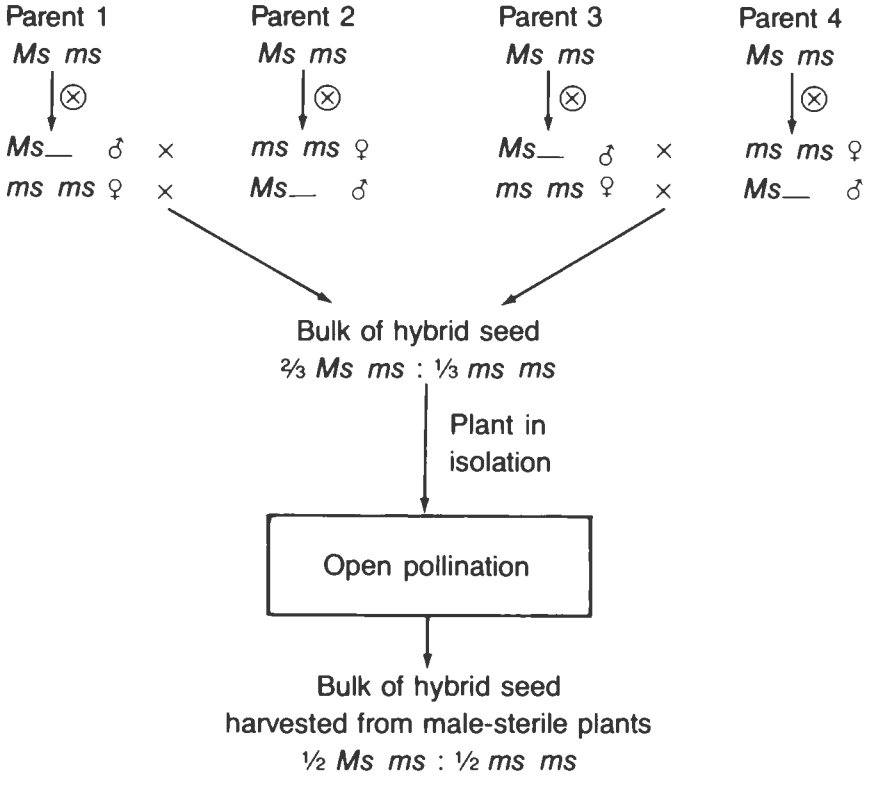


Figure 16-2 Formation of a random-mated population segregating for genetic male sterility by use of four parents heterozygous for the *ms* allele.

of the male-sterile parent for alleles other than *ms* will be 12.5 percent, the population will be developed in the fewest number of seasons possible, and three generations of recombination are conducted after backcrossing is completed.

Season 1: Each male-fertile recurrent parent (*MsMs*) used as the male is manually crossed to male-sterile plants of a female parent serving as the source of male sterility. All of the F_1 plants are heterozygous (*Msms*), and 50 percent of their genes are from the male-sterile parent.

Season 2: To recover the cytoplasm of the recurrent parents, the F_1 plants (male) are backcrossed to their recurrent parent. The BC_1F_1 progeny from the backcross include homozygous (*MsMs*) and heterozygous (*Msms*) male-fertile individuals in a 1:1 ratio. The average genetic contribution of the male-sterile parent in the BC_1F_1 generation is 25 percent.

Season 3: The BC_1F_1 plants (1 *MsMs*:1 *Msms*) are manually crossed to their respective recurrent parents. A number of different BC_1F_1 plants are used and enough crosses are made on each to be certain that the *ms* allele is present in the BC_2 generation. The average genetic contribution of the

male-sterile parent to the BC_2F_1 generation is 12.5 percent, the level desired in the final population.

Season 4: The BC_2F_1 plants (*MsMs* and *Msms*) are self-pollinated and each one is harvested separately.

Season 5: Each BC_2F_1 plant is progeny tested. Progeny derived from homozygous male-fertile plants (*MsMs*) are homogeneous for male fertility and are discarded. Progeny derived from heterozygous male-fertile plants (*Msms*) segregate for male sterility.

The segregating progeny are used to make single crosses among the backcross-derived parents. A single cross is made by crossing male-fertile plants (1 *MsMs*:2 *Msms*) of the male parent onto male-sterile plants of the female parent. Each single cross is harvested separately, and similar seed quantities of each are bulked to form a single population.

Season 6: The population is random-mated by planting it in isolation and permitting open-pollination to take place. The population is made up of $^1/_3$ male-sterile (*msms*) and $^2/_3$ male-fertile (*Msms*) individuals. Seed is harvested from each male-sterile plant individually and a similar quantity of seed from each is bulked for the next season. The seeds of the population are $^1/_2$ *Msms* and $^1/_2$ *msms*.

Season 7: The next generation of recombination is conducted by planting the seed of the population in isolation, allowing open-pollination to occur, harvesting the seed from each male-sterile plant individually, and bulking a similar quantity of seed from each plant. The seed harvested from male-sterile plants is $^1/_2$ *Msms* and $^1/_2$ *msms*.

Each additional generation of recombination is done in the same manner as in season 7. The segregation ratio in the population will be 1 *Msms*:1 *msms*.

The backcrossing procedure described above requires hand emasculation of male-fertile plants used as the female. An alternative procedure is to self the F_1 plants each backcross generation and cross the recurrent parent to male-sterile plants in the F_2 progeny. For example, in season 1 each male-fertile (*MsMs*) parent is mated to male-sterile (*msms*) plants of the donor. In season 2 the F_1 plants (*Msms*) are self-pollinated. In season 3 the F_2 progeny are grown and male-sterile (*msms*) individuals are crossed to the recurrent parent to obtain BC_1F_1 seed. The procedure is continued by self-pollinating the BC_1F_1 plants in season 4. This procedure facilitates crossing but takes more seasons to complete than the one just illustrated (Fig. 16-1).

Population development can involve conventional backcrossing, in which the same recurrent parent is used each backcross generation (Fig. 16-1). An alternative is to vary the matings for the male-fertile parents during each generation of crossing. For example, the F_1 could be *msms* × parent A and the BC_1F_1 could be (*msms* × parent A) × parent D. Each male-fertile parent would be used in a similar number of crosses.

There is considerable diversity among breeders in the number of backcross

generations, if any, used in developing populations and in the number of intermating generations conducted before selection begins. Starkes and colleagues (1976) developed a sorghum population KP2BR by crossing 11 male-fertile parents with greenbug resistance to a population KP1BR that had the male-sterile gene antherless (*al*). Three generations of random mating were conducted before selection began. KP2BR had the cytoplasm of KP1BR and 50 percent of its genes came from the male-sterile donor parent, because no backcrosses were made to the 11 male-fertile parents. Gardner (1972) described the development of a sorghum population in Nebraska that involved transferring a male-sterile allele from Coes germplasm to male-fertile lines by backcrossing, then intercrossing the backcross progeny.

Doggett (1972) described the development of sorghum populations in Uganda. A group of distinct sorghum cultivars was crossed to a source of the ms_3 gene for male sterility, and the single crosses were mated to other cultivars. This provided a final population with 25 percent of its genes from the male-sterile source. F_1 seeds from the three-way crosses were mixed together in equal amounts. In the F_2 generation, male-sterile segregates were selected and their seed was bulked. Three generations of random mating were conducted before selection was begun.

Burton and Brim (1981) developed a soybean population with the male-sterile gene ms_1. Ten male-fertile lines were each crossed to male-sterile plants (ms_1ms_1) to form 10 single-cross populations. An equal quantity of seed from five male-fertile F_1 plants (Ms_1ms_1) from each of the 10 single crosses were bulked. The cycle 0 population had 50 percent of its genes from the male-sterile source, and no random mating was conducted before selection was begun.

The use of genetic male sterility for development of two populations of barley was described by Suneson (1945). One population, designated Composite Cross XIV, was produced by crossing each of the eight leading barley cultivars in California to male-sterile plants of a donor parent, C.I. No. 5368-I, and compositing the F_1 seed. A second population, designated Composite Cross XV, was derived by mating male-sterile plants of the donor parent to 625 male-fertile plants from the F_2 and F_3 generations of three Composite Cross populations, then bulking F_2 seed from the 625 single crosses. Suneson indicated that both F_2 populations would be grown in isolation at normal seeding rates. Open-pollinated seed from male-sterile plants would be harvested and their seed bulked. He suggested that after about three seasons of random mating under competitive conditions, selection could be practiced among male-fertile individuals for yield and other characteristics.

UTILIZATION OF THE POPULATION

A number of methods of recurrent selection can be used with genetic male sterility to improve a population. One principle applies to all methods: male-sterile plants must be tagged at the time of pollination if they are indistinguishable from male-

fertile ones at maturity. In species with effective wind pollination, such as sorghum, seed set on male-sterile plants may be complete, and these plants cannot be differentiated from male-fertile plants at maturity. Male-sterile plants of some species, such as soybean, can be readily identified because they have incomplete seed set and the plants remain green after male-fertile ones are mature.

Individual Plant Selection

Recurrent phenotypic selection involves the identification and recombination of superior individual plants in a population:

Season 1: A population (cycle 0) segregating for genetic male sterility (*Msms*:*msms*) is planted in isolation. Selection is practiced and the superior male-sterile plants are identified. A similar quantity of seed from the selected individuals is bulked to form the cycle 1 population.

Season 2: The cycle 1 population is grown and the plants segregate for male sterility (1 *Msms*:1 *msms*). Selection is conducted as described for season 1 to obtain the cycle 2 population. All subsequent cycles are conducted in the manner described for season 1.

In some self-pollinated species, seed set on male-sterile plants is not complete. For recurrent phenotypic selection to be effective in such cases, it is important that expression of the character under selection not be influenced by amount of seed set. For example, only partial seed set is obtained on male-sterile soybean plants. Selection for seed yield among such plants would probably measure the rate of accidental cross-pollination instead of the genetic potential of the plants for yield.

Selection can be practiced before or after pollination occurs. Selection before pollination provides control of both the female and male parents. Only the female parent is controlled if selection is practiced after pollination.

The number of plants available for selection and the frequency of plants retained should be large enough to include the desired number of male-sterile individuals. Seed of selected individuals will be used to form the population for the next cycle of selection. For example, assume that a population is segregating in the proportion 50 percent male fertile (*Msms*):50 percent male sterile (*msms*), the selection intensity is 25 percent, and the number of selected male-sterile plants to be harvested is 200. The initial population size required would be 1600 plants, computed as follows:

$$\text{Initial population size} = \frac{\text{number of selected male-sterile plants}}{\text{selection intensity} \times \text{frequency of male-sterile plants}}$$

$$1600 \text{ plants} = \frac{200}{0.25 \times 0.50}$$

Selection can be practiced for plant or seed characteristics. When selection is for seed characters, part of the seed from each male-sterile plant is used for analysis and part is retained in storage. Seed of superior plants is taken from storage and bulked to form the improved population.

Random pollination of single plants does not occur with open-pollination because adjacent plants are more likely to be mated than those separated by some distance. Furthermore, microenvironmental conditions may not be uniform throughout the test site, so that plants in one area of a field may be favored over those in another. The effect of these factors can be reduced by subdividing the area into blocks and selecting a similar number of superior plants from each block, as suggested by Gardner (1961) (Chap. 15). For example, assume that 100 superior individuals are to be selected. The plants in the test site could be divided into 100 subblocks and one superior plant chosen from each.

For a character that is evaluated after pollination, Doggett (1968) indicated that selection among open-pollinated male-sterile plants can be alternated with selection among self-pollinated male-fertile plants (Fig. 16-3):

Season 1: Selection for the female parent is conducted in season 1. A population (cycle 0) segregating for genetic male sterility (*Msms*:*msms*) is planted in isolation. Desirable male-sterile plants are selected and harvested individually. A similar quantity of seed from each plant is bulked for planting the next season.

Season 2: Selection for both parents is accomplished in season 2. The population obtained in season 1 is planted and segregation occurs for heterozygous male-fertile (*Msms*) and male-sterile plants. Desirable male-fertile plants are selected and selfed seed is harvested from each selection. A similar quantity of selfed seed from each plant is bulked to form the cycle 1 population.

Season 3: The next cycle of selection begins. The cycle 1 population is planted in isolation. The plants segregate (1 *MsMs*:2 *Msms*:1 *msms*) because they were derived from heterozygous male-fertile individuals. Each cycle of selection is conducted by repeating the procedures of seasons 1 and 2.

The advantage of this procedure is that the potential genetic gain is greater than when selection is practiced among male-sterile plants every season. Selection among self-pollinated male-fertile plants provides control of both parents, compared with control of only the female parent when selection is practiced among male-sterile individuals after pollination. Doggett (1972) utilized the above procedure to conduct recurrent selection in sorghum populations. In each season, 200,000 plants per hectare of a population were grown. Each population was divided into subblocks of 200 plants and the best individual was chosen from each on the basis primarily of yield. Selection only among male-sterile plants resulted in an average yield increase for eight populations of 7 percent after three cycles of selection. For the same populations, there was a yield

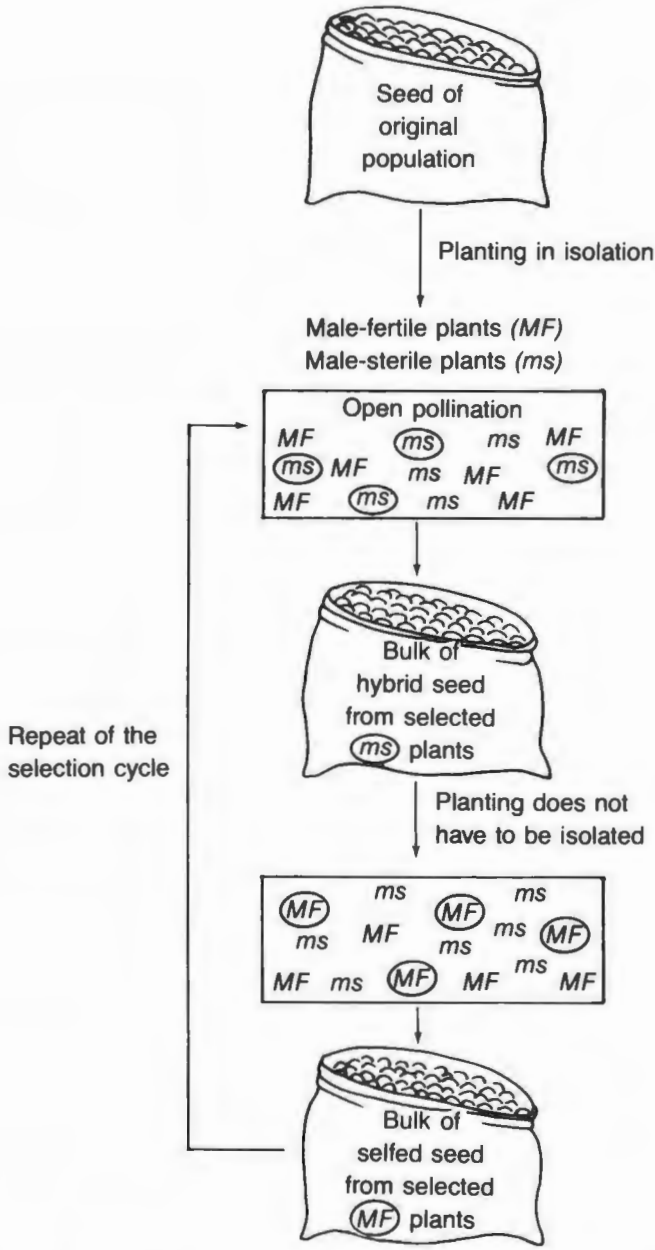


Figure 16-3 Recurrent phenotypic selection among male-sterile and male-fertile plants in alternating seasons, as described by Doggett (1968).

increase for alternating selection of 11 percent after one and a half cycles, i.e., male-sterile plants selected in season 1, male-fertile plants selected in season 2, and male-sterile plants selected in season 3.

An example of repeated cycles of selection among male-sterile individuals was reported by Burton and Brim (1981). They reported on the results of recurrent selection among male-sterile plants for increased oil percentage in soybean seed. The open-pollinated population segregating for male sterility was divided into 28 subblocks, and the plants with the highest oil percentage in each subblock were bulked to form the new population. The mean oil percentage of the population increased from 18.8 to 19.7 percent after three cycles of selection.

Selection Among Half-Sib Families

The progeny of male-sterile plants that have been open-pollinated constitute a half-sib family. Recurrent half-sib selection can be practiced among families using seed from the male-sterile plants (Fig. 16-4):

Season 1: A population (cycle 0) segregating for genetic male sterility (*Msms*, *msms*) is planted in isolation. Male-sterile plants are harvested individually, each representing a half-sib family. Part of the seed from each plant is put in storage and the other part is used for testing in season 2.

Season 2: The half-sib families are evaluated in replicated tests and the superior ones are selected. Remnant seed of the superior half-sib families is removed from storage and a similar quantity of seed from each is bulked to form the cycle 1 population.

Season 3: The next cycle of selection begins. The cycle 1 population is planted in isolation. Plants in the population segregate in the ratio 1 *Msms*:1 *msms*. The procedures described for selection in seasons 1 and 2 are repeated for each subsequent cycle of selection.

The progeny from a male-sterile individual segregate in the ratio 1 male-fertile (*Msms*):1 male sterile (*msms*); therefore, the feasibility of selection will depend on the extent to which male sterility affects expression of the character under selection. For species that do not have complete seed on male-sterile plants, the effectiveness of selection among half-sib families may be affected for some characters.

If sufficient seed is not available from a male-sterile plant for evaluation of the progeny, a generation of seed increase may be required. In self-pollinated species, the male-fertile plants in each half-sib family will produce selfed seed during the generation of increase. Unless each half-sib family is grown in isolation, the male-sterile plants will be contaminated by pollen from plants of different families. To avoid contamination, male-sterile plants should be discarded and the seed of male-fertile plants in a family should be harvested in bulk for testing.

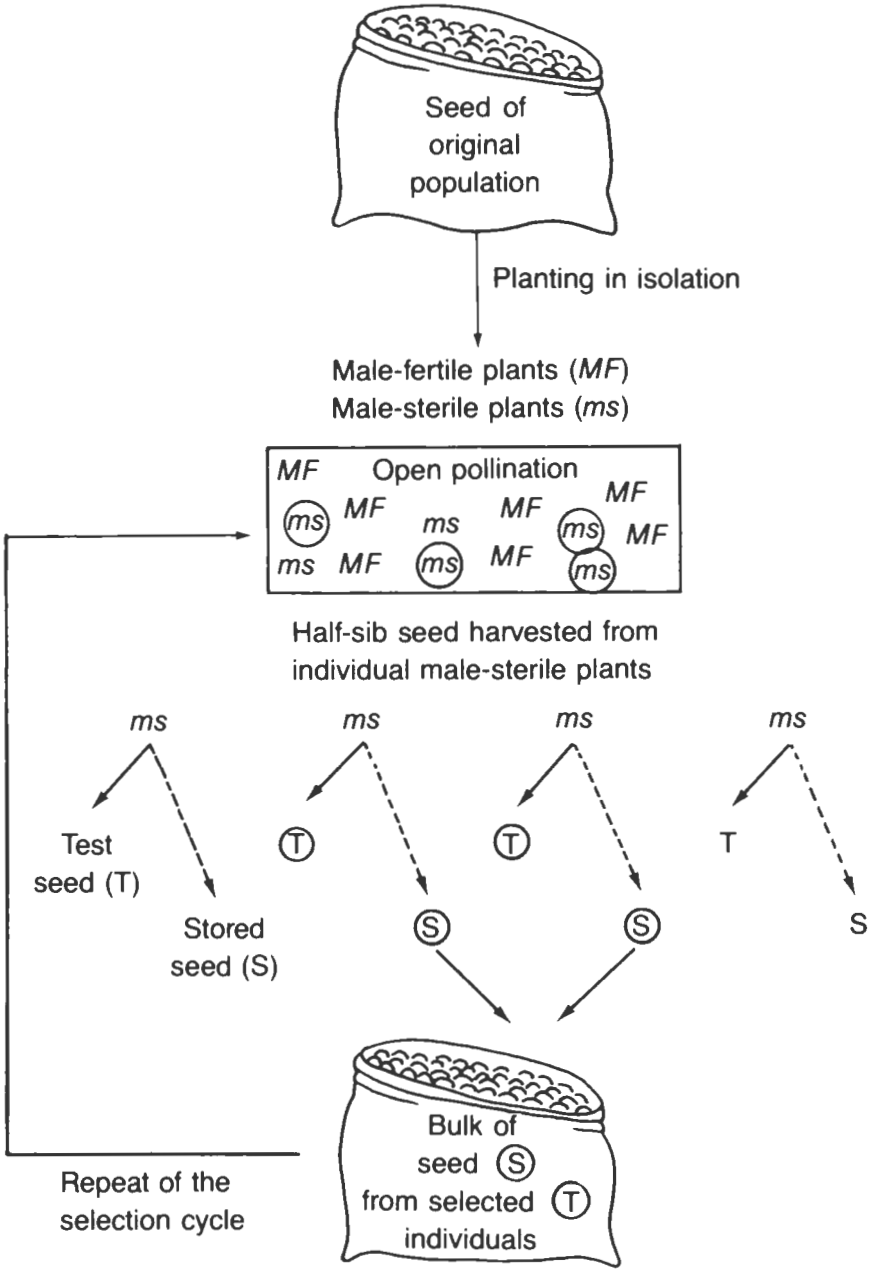


Figure 16-4 Recurrent selection among half-sib families in a population segregating for genetic male sterility.

When a generation of seed increase is needed, the seed of superior half-sib families used to form the population for the next cycle of selection can be derived from two sources.

1. Part of the seed harvested from each male-sterile plant in season 1 can be put in storage and the remaining part used for the seed increase in season 2. The seed of selected families is taken from storage and bulked to form the new population. The new population will segregate in the ratio 1 *Msms*:1 *msms*.
2. Part of the seed from the male-fertile plants harvested in the generation of increase can be put in storage and the remaining seed used for testing. The new population would be formed by bulking seed from storage of selected families. The new population will segregate in the ratio (1 *MsMs*: 2 *Msms*:1 *msms* because the seed is derived from heterozygous male-fertile plants in the generation of increase.

Selection Among and Within Half-Sib Families

For some characters it is possible to select among half-sib families, then select on a single-plant basis within superior families.

To begin the selection program, a population (cycle 0) segregating for genetic male sterility (*Msms, msms*) is planted in isolation. The male-sterile plants are harvested individually, each representing a half-sib family.

Season 1: The half-sib families are evaluated in replicated tests. One replication of the test or a separate planting of the entries is planted in isolation. At that site, rows of the half-sib families are alternated with rows of a pollinator. The pollinator represents a composite of seed of all the half-sib families in the test. Male-fertile plants within the female rows are discarded at the time of pollination. Superior families are selected in the replicated test, and superior male-sterile plants are selected within them. Each selected male-sterile plant is part of the cycle 1 population and represents a half-sib family for the next cycle of selection.

Season 2: The next cycle of selection begins. The procedures for selection used in season 1 are repeated for all subsequent cycles of selection.

It is possible to complete a cycle of selection in one season, if selection within families is among male-sterile female plants that have been pollinated by unselected males. The procedure is the same in principle as modified ear-to-row selection in maize, as described by Lonnquist (1964).

The selection process requires two seasons per cycle if both the female and male parent are selected. Selection can be based on male-fertile plants within the superior half-sib families.

Season 1: A population (cycle 0) segregating for genetic male sterility (*Msms, msms*) is planted in isolation. Male-sterile plants are harvested individually, each representing a half-sib family.

Season 2: The half-sib families are evaluated, the superior families are identified, and superior self-pollinated male-fertile (*Msms*) plants are selected within them. A similar quantity of selfed seed from each selected plant is bulked to form the cycle 1 population.

Season 3: The next cycle of selection begins. The cycle 1 population planted in isolation segregates 1 *MsMs*:2 *Msms*:1 *msms* because the seed was derived from selected self-pollinated male-fertile plants that were heterozygous (*Msms*) for the male-sterility allele. The procedures described for selection in seasons 1 and 2 are repeated for each subsequent cycle of selection.

When selection among and within families is conducted, plants are selected from within one plot of each superior half-sib family. The plots may be part of a replicated test or may be a separate planting. If the superior families can be identified before harvest, superior plants can be selected within them. If selection of the superior families cannot be done until after harvest, plants must be harvested individually from one plot of every family.

There is a possible disadvantage of selecting male-sterile plants within families, instead of male-fertile ones. Male-fertile plants may pollinate adjacent male-sterile plants within a row of a half-sib family more often than the intended male-fertile parent grown in an adjacent row. Eliminating male-fertile plants within the rows can minimize the problem, but may be time-consuming.

Selection Among Plants and Within Half-Sib Families

It is possible to select among male-sterile plants in a population followed by selection among male-fertile plants within their half-sib progeny. Burton and Brim (1981) conducted three cycles of recurrent selection for high oil percentage in soybean seed. They subdivided a population segregating for genetic male sterility into 28 subblocks, each with 12 male-sterile plants. A plant in each subblock was chosen, then half-sib progeny of the 28 selections were grown. The male-fertile plant with the highest oil percentage within each half-sib progeny was selected to form the new population. Oil percentage increased from 18.8 to 19.9 percent after three cycles of selection.

Selection Among Full-Sib Families

Full-sib families are formed by mating two individual plants within a population. When genetic male sterility is employed, a full-sib family is formed by manually mating a male-fertile plant to a male-sterile one:

Season 1: A population (cycle 0) segregating for genetic male sterility (*Msms*:*msms*) is planted. Full-sib families are formed by manually crossing a male-sterile plant to a male-fertile one. Part of the seed of each family is put in storage to be used for recombination of selected families in season 3 and the other part is used for testing in season 2.

Season 2: The full-sib families are tested and superior ones are selected. An equal quantity of seed of each selected family is taken from storage and bulked.

Season 3: The bulk of the selected full-sib families is planted in isolation and the plants segregate in the ratio 1 *Msms*:1 *msms*. Seed is harvested from male-sterile plants individually and a similar quantity from each is bulked to form the cycle 1 population.

Season 4: The next cycle of selection begins. The cycle 1 population is planted and full-sib families are formed. Each cycle of selection is conducted by repeating the procedures of seasons 1 to 3.

The requirement for manual hybridization limits the desirability of full-sib selection for population improvement when genetic male sterility is utilized.

Selection Among Selfed Progeny

Recurrent selection among $S_{0:1}$ lines or more advanced generations of selfing can be practiced with the use of genetic male sterility. Selection can involve progeny segregating for male sterility or progeny that are uniform for male fertility.

The simplest and most rapid procedure is selection among $S_{0:1}$ lines segregating for male sterility (Fig. 16-5). Each cycle of selection requires three seasons, if only one season is used for testing.

Season 1: An S_0 population (cycle 0) segregating for male sterility is grown and male-fertile (*Msms*) plants are harvested individually. Each plant will be tested as an $S_{0:1}$ line in season 2. Part of the S_1 seed is used for testing and part is put in storage to be used for intercrossing selected $S_{0:1}$ lines in season 3.

Season 2: The $S_{0:1}$ lines are evaluated and the superior lines are selected. An equal quantity of remnant S_1 seed from storage for each selected line is bulked.

Season 3: The seed bulk is grown in isolation and the plants segregate in the ratio 1 *MsMs*:2 *Msms*:1 *msms*. The male-sterile plants are harvested individually and an equal quantity of seed from each is bulked to form the cycle 1 population.

Season 4: The next cycle begins. Male-fertile plants from the cycle 1 population are selected. Each cycle of selection is conducted in the manner described for seasons 1 to 3.

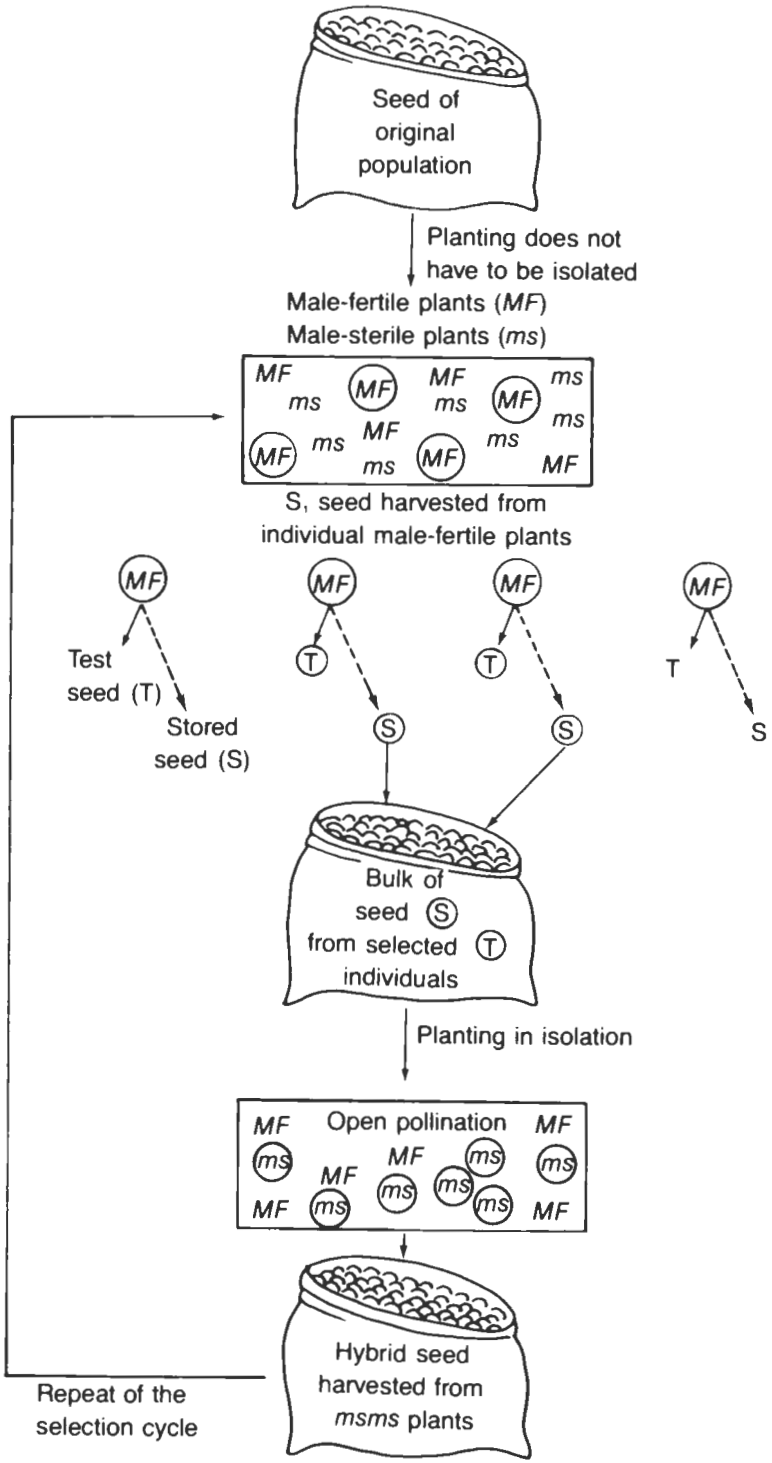


Figure 16-5 Recurrent selection among S_0-derived lines by use of progeny segregating for male fertility and male sterility.

Doggett (1972) evaluated $S_{0:1}$ lines in sorghum populations in Uganda. He chose self-pollinated heads of male-fertile plants from the populations and tested the $S_{0:1}$ lines in a 13×13 triple lattice. Averaged over populations, he observed an average yield increase of 25 percent after one cycle of selection.

Two sources of seed are available for intercrossing the selected lines, remnant S_1 seed, as described in the illustrations, or seed of male-fertile plants harvested from the $S_{0:1}$ test. Bulk seed harvested from the $S_{0:1}$ test is likely to be impure if it includes seed from open-pollinated male-sterile plants and self-pollinated male-fertile plants. Some seed on the male-sterile plants is likely to have developed from pollen derived from other $S_{0:1}$ lines.

If adequate seed for testing cannot be obtained from a single S_0 plant, a generation of seed increase may be needed. Unless each $S_{0:1}$ line is grown in isolation, only seed from self-pollinated male-fertile S_1 plants of a line should be harvested in bulk. Part of the harvested seed of each line would be put in storage to use for intercrossing selected lines after testing was completed. Segregation within each line during testing and in selected lines used for intercrossing would be in the ratio 3 *MsMs*:2 *Msms*:1 *msms*.

The segregation for male sterility in replicated tests may be unacceptable for effective evaluation of a character. Figure 16-6 illustrates a procedure for the evaluation of S_0-derived lines that are homogeneous for male fertility. The following illustration is of a procedure that could be used to evaluate S_2-derived lines using homogeneous male-fertile progeny. Homogeneous male-fertile lines can be identified readily by the use of progeny testing. The primary difficulty is that a homogenous male-fertile line lacks the male-sterile allele needed for intercrossing to begin the next cycle of selection. Because the line itself cannot be used for open-pollination, remnant seed of the heterozygous *Msms* individual from which the line was selected must be used. The use of homogeneous male-fertile progeny for testing markedly increases the complexity and number of seasons required for each cycle.

Season 1: A population (cycle 0) segregating for genetic male sterility is planted in isolation. The male-sterile plants are harvested individually. The progeny from each plant will be maintained as a family during the generations of selfing.

Season 2: The S_0 progeny of each family are grown and a male-fertile (*Msms*) S_0 plant is harvested.

Season 3: The $S_{0:1}$ line of each family is grown and the plants segregate (1 *MsMs*:2 *Msms*:1 *msms*). Enough male-fertile S_1 plants are harvested individually from each family to be adequately certain that one is heterozygous.

Season 4: The S_2 progeny from each S_1 plant are grown and one progeny row of each family that segregates for male sterility: (1 *MsMs*:2 *Msms*: 1 *msms*) is selected. Enough male-fertile S_2 plants are harvested individually from the selected segregating rows to be adequately certain that one is heterozygous. Part of the S_3 seed from each S_2 plant is put in

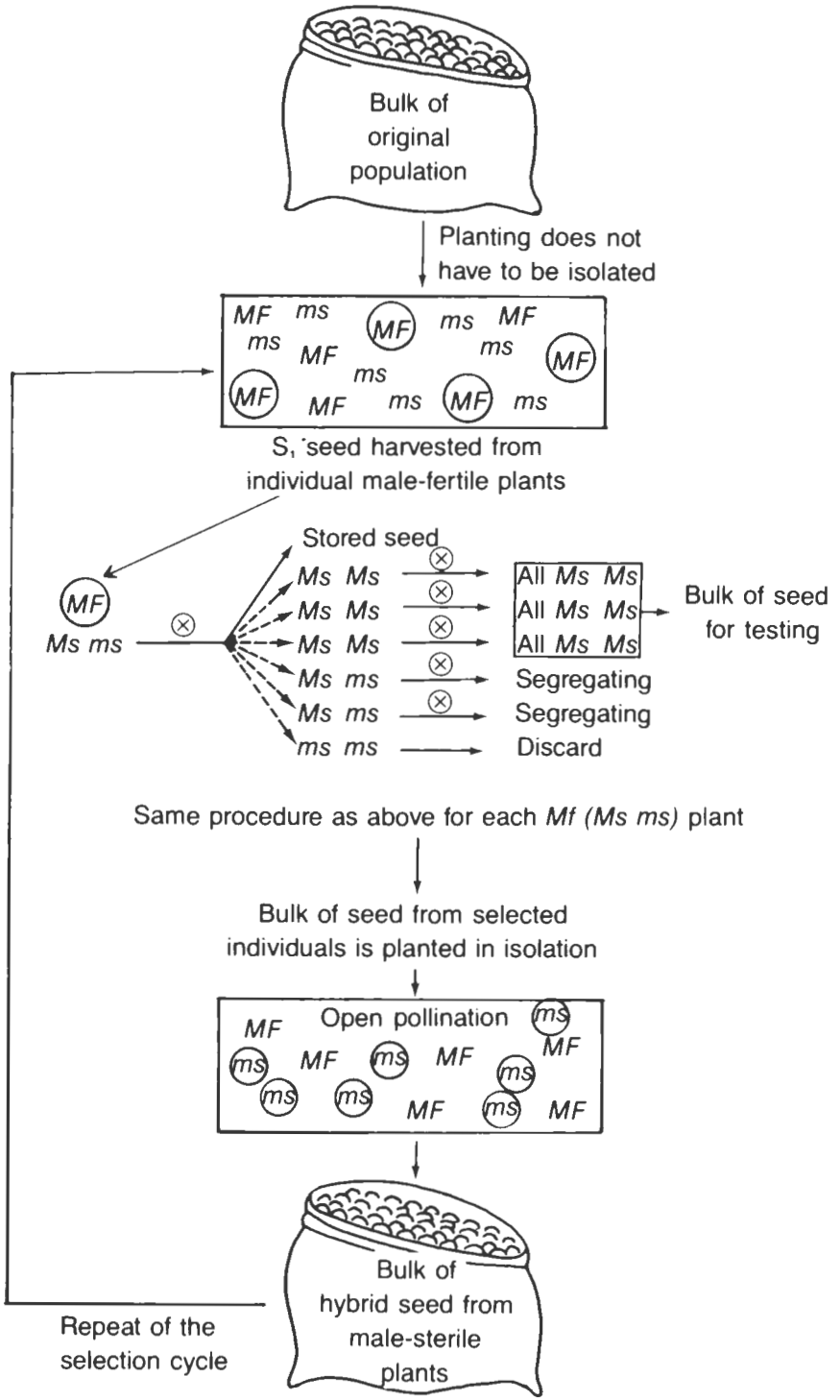


Figure 16-6 Recurrent selection among S_0-derived lines by use of homogeneous male-fertile progeny.

storage to be used for intercrossing selected lines in season 8. The other part of the seed is used to plant a progeny row in season 5.

Season 5: The $S_{2:3}$ lines are grown and lines are selected that segregate for male sterility (3 *Ms*__: 1 *msms*). Enough male-fertile S_3 plants are harvested individually from the selected segregating rows to be adequately certain that at least one, and preferably more, are homozygous for male fertility (*MsMs*).

Season 6: The $S_{3:4}$ lines are grown and lines homogenous for male fertility are selected that trace to a particular S_2 plant. The male-fertile $S_{3:4}$ lines that trace to the same S_2 plant are threshed together in bulk to form an $S_{2:5}$ line for evaluation in a replicated test during season 7.

Season 7: A replicated test is conducted to evaluate the $S_{2:5}$ lines. The lines with the best performance are selected, each tracing to a different S_2 plant harvested in season 4. Remnant seed of the S_2 plant from season 4 is taken from storage and a similar seed quantity of each plant is bulked for intercrossing in season 8.

Season 8: The bulk of seed from selected lines is planted in isolation. The plants segregate in the ratio 1 *MsMs*:2 *Msms*:1 *msms*. Male-sterile plants are harvested separately and a similar number of seeds from each is bulked to form the cycle 1 population.

Season 9: The next cycle of selection begins by planting the cycle 1 population in isolation. Each cycle of selection is conducted by repeating the procedures described for seasons 1 to 8.

Natural Selection in Populations for Intrapopulation Improvement

The primary use of populations segregating for genetic male sterility has been for artificial selection by the methods discussed in the previous sections. However, Suneson (1956) proposed a method of breeding that involves natural selection in genetically diverse populations. He referred to the concept as the evolutionary plant breeding method because natural selection was expected to increase the frequency of genotypes that had the highest reproductive capacity.

The procedure for the evolutionary breeding method is to grow a large number of plants of a population in the environment for which new cultivars are being developed. In a self-pollinated species, self-pollination occurs for male-fertile plants and open-pollination for male-sterile plants. The population is harvested in bulk and a sample of the seed is used to plant the next generation. Suneson (1945) suggested that 15 generations of natural selection should take place before an attempt is made to identify superior cultivars in the population.

The role of genetic male sterility for the evolutionary breeding method is to facilitate recombination in the population. There is no selective harvest of male-sterile plants; therefore, the frequency of male-sterile individuals each generation will vary.

INTERPOPULATION IMPROVEMENT

For crops in which hybrid seed is used for commercial production, it may be desirable to improve two different populations simultaneously. The improved populations could be mated to form a cultivar-cross hybrid, or inbred lines extracted from one population could be crossed with superior inbred lines obtained from the other population to form a single-cross hybrid. When cytoplasmic-genetic male sterility is used to produce commercial hybrids, consideration must be given to restorer genes in the populations to be improved (Chap. 34). One population would have only parents with male-fertile cytoplasm and nonrestorer alleles. Inbred lines derived from the population would be used as B lines and cytoplasmic male-sterile A lines would be developed from them by backcrossing. The second population would have parents with male-fertile cytoplasm and restorer alleles for the development of R lines. Genetic male sterility would be incorporated into both of the populations and selection could be practiced independently in the populations using one of the intrapopulation methods. Interpopulation improvement by reciprocal half-sib or reciprocal full-sib selection also could be practiced.

Reciprocal half-sib selection involves the use of two populations, each being the tester for the other. Manual pollination is required to form the half-sib families.

Season 1: Two populations (A and B) are grown that are segregating for male sterility (*Msms:msms*). To form a half-sib family, a male-fertile plant (*Msms*) from one population is used to manually pollinate multiple male-sterile plants in the other population. The seed from the male-sterile plants is bulked to form half-sib seed for progeny testing the male-fertile individual in season 2. Self-pollinated seed from the male-fertile plant is put in storage for use in intercrossing individuals with superior half-sib progeny. At the end of season 1, there are half-sib progeny available for male-fertile plants from both population A and population B.

Season 2: The half-sib families from population A and population B are evaluated and the best families are selected independently for each population. An equal quantity of seed is taken from storage and bulked for each male-fertile plant of population A that produced a superior half-sib family. The same is done independently for selected plants of population B.

Season 3: The seed bulks of populations A and B are grown in separate isolations. The plants in the populations segregate 1 *MsMs*:2 *Msms*:1 *msms* because they were derived from heterozygous male-fertile individuals. Each of the populations are open-pollinated, male-sterile heads are harvested individually, and a similar amount of seed from each is bulked to form cycle 1 populations of A and B.

Season 4: The next cycle begins. The two populations are planted and half-sib families are formed for each. Each cycle of selection is conducted by repeating the procedures for seasons 1 to 3.

Reciprocal full-sib selection involves crossing the selfed progeny of heterozygous *Msms* individuals from two populations. Direct plant-to-plant crosses for reciprocal full-sib selection are not possible with genetic male sterility. A male-fertile individual can be crossed to a male-sterile one, but the male-sterile individual cannot be self-pollinated.

Season 1: Two populations (A and B) are grown that are segregating for male sterility (*Msms:msms*). Male-fertile S_0 plants are selected independently in the two populations. Part of the S_1 seed from each plant is put in storage to be used for intercrossing selected individuals. The other part of the seed is used to form full-sib families in season 2.

Season 2: The S_1 progeny of plants from population A are paired with the S_1 progeny of plants from population B. To form a full-sib family, male-fertile plants of an $S_{0:1}$ line from population A are used to manually pollinate male-sterile plants of an $S_{0:1}$ line from population B, and vice versa. The seed from the reciprocal crosses is bulked to form a full-sib family for testing in season 3.

Season 3: The full-sib families are tested and the superior ones are selected. To prepare for intercrossing in season 4, S_1 seed is taken from storage for each S_0 plant from population A that was represented by a selected full-sib family. An equal quantity of S_1 seed from each plant is bulked. The same procedure is used to prepare a seed bulk for intercrossing selected members of population B.

Season 4: The seed bulks of populations A and B are grown in separate isolations for open-pollination. There are three-fourths male-fertile (*Ms__*) and one-fourth male-sterile segregates in each population. In each population, male-sterile plants are harvested individually and an equal quantity of seed of each is bulked to form the cycle 1 populations of A and B.

Season 5: The next cycle begins. The two populations are planted and male-fertile plants are selected. Each cycle is conducted by repeating the procedures used in seasons 1 to 4.

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