CHAPTER THIRTY-FOUR

Development of Hybrid Cultivars

Hybrid cultivars represent the F_1 progeny of matings that may involve inbred lines, clones, or populations. The most common type of hybrid cultivar is produced by crossing two or more inbred lines; therefore, the development of inbred lines for use in hybrids will be the emphasis of this chapter. The development of inbred lines from a segregating population has six aspects: (a) formation of a segregating population, (b) inbreeding of the population to an adequate level of homozygosity, (c) evaluation of the performance of a line per se, (d) evaluation of the general combining ability of a line, (e) evaluation of a line in potential commercial hybrids, and (f) preparation of breeder seed of an inbred line. Alternative procedures are available for each phase of the development process.

FORMATION OF A SEGREGATING POPULATION

Natural Populations or Open-Pollinated Cultivars

The populations used for the initial inbred line development of a cross-pollinated species are natural populations or open-pollinated cultivars that evolved from selection by farmers using natural populations. Such populations are an important source of variability in the country of origin and in other countries with similar environmental conditions.

Gamete Selection

A method of utilizing open-pollinated populations to form segregating populations with a high frequency of desirable individuals was proposed by Stadler

(1945). He indicated that a superior individual in an open-pollinated population results from the union of superior male and female gametes. The probability that two superior gametes will unite to form a superior individual is the square of the frequency of superior gametes. For example, if 10 percent of the gametes are superior, 0.1^2 or 1 percent of the zygotes (individuals) should be superior. In a population in which the frequency of superior individuals is low, selection of gametes instead of zygotes may increase the probability of identifying useful genes. The gamete selection method involves the sampling of gametes from a population by crossing the population to an elite inbred line. Superior individuals from the cross are identified by testcross evaluation. The selfed progeny of superior individuals represent a population from which inbred lines can be developed.

Season 1: A segregating population is crossed to an inbred line. Each F_1 seed from the cross represents the mating of a different gamete of the population with gametes from the inbred. Assuming that every gamete of the inbred is the same, the genotypes of the F_1 seeds differ only by the genes of the gametes from the population.

Season 2: F_1 seeds from the population-inbred cross are planted, and individual plants are self-pollinated and crossed to a tester. The self-pollinated F_2 seeds of each F_1 individual are put in storage for use in season 4. The testcross seed from each F_1 individual is used for replicated trials in season 3.

The inbred is crossed to the tester for use as a check in the replicated trials of season 3.

Season 3: A replicated trial is conducted that includes the testcross progeny of the F_1 individuals being evaluated and the inbred × tester hybrid. F_1 individuals with testcross performance superior to the inbred × tester hybrid are selected.

Season 4: The self-pollinated F_2 seed of each superior F_1 individual that was produced in season 2 is an F_2 population. Inbred line development is carried out in the population in the same manner as populations formed by any other procedure.

The germplasm needed to conduct gamete selection includes a segregating population, an inbred line, and a tester. The inbred line should be highly desirable, because it will contribute half of the alleles to the populations developed by gamete selection. Selection of a tester for gamete selection involves the same considerations as for any testcross progeny evaluation.

The advantage of gamete selection is that an F_2 population formed from a population × inbred cross is likely to provide a higher frequency of desirable inbred lines than would be obtained by inbreeding the population per se. The disadvantage of the method is that the amount of genetic variability in the population × inbred cross is more limited than in the population per se because half of the genes are contributed by the inbred.

Crosses between Inbred Lines

Inbred lines are used to develop populations of single crosses, three-way crosses, double crosses, backcrosses, and complex crosses. They also are used to produce synthetic cultivars that can be used as populations for inbred line development.

Selection of parents for a population may involve consideration of the heterotic relationships among available genotypes and cytoplasmic-genetic interactions involved in hybrid seed production.

Heterotic Relationships. Performance of a hybrid is a function of the amount of heterosis expressed in the cross between two parents. Heterosis generally increases as the genetic diversity between the parents increases; therefore, breeders of hybrid cultivars sometimes attempt to maintain two germplasm pools that provide the desired heterosis in crosses between them. Maize breeders in the U.S. Corn Belt have observed that good heterosis is obtained when parents derived from Iowa Stiff Stalk germplasm are crossed to those derived from Lancaster germplasm. An attempt is made to develop breeding populations with lines derived from Iowa Stiff Stalk and independent populations involving lines derived from Lancaster to maintain the genetic diversity between the two germplasm pools.

Cytoplasmic-Genetic Relationships. Production of hybrid cultivars by the use of cytoplasmic-genetic male sterility requires appropriate lines with restorer genes (R lines), with male-fertile cytoplasm and nonrestorer genes (B or O lines), and with male-sterile cytoplasm and nonrestorer genes (A lines). Breeding populations are established for development of nonrestorer lines (B or O lines) from which cytoplasmic male-sterile females are prepared. Separate populations are used to develop the restorer lines (R lines) used as males in hybrid seed production. Development of separate restorer and nonrestorer populations also helps to maintain genetic diversity between two germplasm pools. Populations developed from crosses among parents and restorer genes are referred to as R populations, and those developed from crosses among nonrestorer lines are referred to as B or O populations.

Populations Derived by Recurrent Selection

A population improved by recurrent selection can be useful for inbred line development. Lines evaluated as part of a recurrent selection program can be used as parents to form a new population and can be further inbred for possible use in a hybrid.

INBREEDING OF THE POPULATION

The inbred lines used to produce hybrid cultivars are developed by self-pollination or sib mating. Self-pollination is most frequently used because homozygosity is achieved more rapidly than when plants within a line are sib mated (Chap. 8).

Amount of Inbreeding

A breeder must make a number of decisions in developing a program of inbred line development. One decision is the amount of inbreeding that will be conducted before a line is considered adequately homozygous. This is an important consideration, particularly for cross-pollinated species, because it will affect the vigor of the parent, the ability to maintain its genetic integrity, and the ease with which it is managed in a seed production field.

For cross-pollinated species, the vigor of inbred lines from a population is inversely proportional to the amount of inbreeding. Vigor of a line includes to its ability to produce seed as a female parent or pollen as a male parent. For economical seed production, particularly of single-cross hybrids, the vigor of an inbred parent is a major consideration. It is an advantage, therefore, to use only a limited amount of inbreeding in developing parents for commercial hybrids.

The disadvantage of limited inbreeding is the difficulty that may arise in maintaining the genetic integrity of an inbred parent during multiple generations of seed production. An inbred line derived from plants in an early generation of inbreeding is more likely to be heterogeneous than lines derived from highly inbred plants. A line may be adequately homogeneous with respect to visual uniformity while possessing genetic variability for quantitative characters. When the heterogeneous inbred parent is grown for seed production in diverse environments for a number of years, natural selection can alter its genetic makeup. As a result, the characteristics of such a parent produced for several years in two locations may become different.

Heterogeneity of an inbred also is a consideration in seed production fields. It is easier for inexperienced persons to rogue off-type plants if the inbred parent has a high degree of genetic uniformity rather than variability for visual characteristics.

Methods of Inbreeding

The methods that can be used to develop inbred lines include pedigree, bulk, single-seed descent, and early-generation testing. The choice of a method or combination of methods is influenced by the environments that are available to the breeder. The relationship between method of inbreeding and available environments can be examined with a breeding program for hybrids to be grown in temperate climates. It is common for breeders to use off-season nurseries in tropical environments to reduce the length of time for inbred line development. The growth of plants and expression of characters in off-season nurseries often are different than when the plants are grown in their area of adaptation. In such cases, the nurseries are useful for inbreeding but not for selection. Inbred line development would be delayed if the pedigree or bulk method was considered for the breeding program. In the off-season nursery, the selection necessary for the pedigree method could not be conducted, and the bulk method would be hampered by atypical performance of genotypes. The evaluation phase of early-generation testing could not be done in the off-season nursery, but the nursery could be used to produce the testcross seed necessary for the replicated trial. The single-seed descent method could be used effectively in the off-season nursery to permit rapid inbred line development. A combination of methods often provides an appropriate inbreeding strategy when certain environments can be used only for inbreeding.

EVALUATION OF COMBINING ABILITY

General Combining Ability

In a hybrid development program, the objective is to identify a new line that, when crossed with other parents, will produce hybrids with superior performance. If resources were unlimited, it would be best to test immediately each new inbred in combination with every other inbred with which it could be a parent in a hybrid cultivar. This is not feasible because of the large number of single-cross combinations that would have to be tested. For example, the number of single crosses possible among n parents is equal to $[n\,(n-1)]/2$. Evaluation of 1000 lines in all possible single-cross combinations would involve $[1000\,(999)]/2 = 499{,}500$ hybrids, an impractical number of entries to test in any breeding program. The breeder must identify a limited number of lines with sufficient genetic potential before their evaluation in specific hybrid combinations.

The first step in evaluating the potential of new lines is to cross them to a common parent and compare the performance of their hybrids. The common parent is referred to as the tester and the hybrids produced are referred to as testcrosses or topcrosses. The tester is the same for all new lines being evaluated; therefore, differences in performance among the hybrids reflect differences in the general combining ability of the lines. General combining ability refers to the average performance of a line in crosses with other parents. Specific combining ability is the performance of a line in a cross with a specific parent. For example, if line P1 is crossed to parents P2, P3, and P4, the average performance of hybrids P1 × P2, P1 × P3, and P1 × P4 would reflect the general combining ability of line P1. The specific performance of any of three hybrids reflects the specific combining ability of line P1 with one of the other parents.

The testers used to determine general combining ability in the early years of hybrid development were heterogeneous cultivars, populations, or crosses, referred to as broad-base testers. A heterogeneous tester was used to represent the array of genes that a line could be associated with if it were crossed to individual parents in a series of single crosses. The performance of a line in association with such an array of genes gave a measure of its average ability to combine with other inbred parents.

The testers most commonly used today for the first evaluation of combining ability of a line are the inbreds with which it would most likely be crossed to produce a commercial hybrid. For species in which commercial hybrids are single crosses, the tester is an inbred line that is widely used for hybrid seed production. Three-way hybrid cultivars are common in sugar beet, and the testers used to evaluate general combining ability are single-cross hybrids used to produce commercial cultivars. Parents of current cultivars are used as testers because they provide good information about the general combining ability of a line with other potential parents and also provide information on specific combining ability of the line with the tester. The line × tester combination may turn out to be a useful commercial hybrid, which shortens the length of time for hybrid evaluation and release. When a broad-base tester was used in the past, only general combining ability was evaluated for the first 1 or 2 years and then tests of specific hybrid combinations followed.

Only one or two inbred testers are used for the first evaluation of combining ability because the number of testcrosses increases as a multiple of the number of testers. Evaluation of 1000 lines with one tester involves 1000 testcross hybrids, and evaluation of 1000 lines with two testers involves 2000 hybrids. When the resources for testing are fixed, the breeder must choose between evaluating more lines with less precision with one tester or fewer lines with more precision with two or more testers.

Lines that have good performance in the first evaluation are advanced to tests involving more testers and eventually to evaluation in specific hybrid combinations. With each year of evaluation, the number of lines decreases and the extensiveness of testing increases for the lines retained.

Specific Combining Ability

The ultimate test for a line is to evaluate its performance as a parent in hybrids that could be used commercially. When broad-base testers are used for determining general combining ability, none of the line × tester combinations is considered to be a potential commercial hybrid. Therefore, there is a clear distinction between the tests for general combining ability and tests of specific parent combinations for evaluation of specific combining ability. The distinction between evaluation of general and specific combining ability is not so clear when the tester for the first evaluation of a new line is a desirable inbred or single cross that eventually may be mated with the new line to produce a commercial hybrid.

Prediction of Three-Way and Double-Cross Hybrid Performance

The evaluation of a group of inbred lines for the production of a three-way or double-cross hybrid is hampered by the number of cross-combinations that are possible. The formulas for determining the number of possible crosses among a group of n parents, excluding reciprocals, are as follows:

$$\text{Number of single crosses} = \frac{n(n-1)}{2}$$

$$\text{Number of three-way crosses} = \frac{n(n-1)(n-2)}{2}$$

$$\text{Number of double crosses} = \frac{n(n-1)(n-2)(n-3)}{8}$$

With only 20 inbred lines, there would be 190 possible single crosses, 3420 three-way crosses, and 14,535 double crosses. The number of possible three-way and double crosses is too large for evaluation; therefore, the number has to be reduced to a more practical number.

Jenkins (1934) developed a method of predicting double-cross performance that has been widely used to identify those combinations of inbred lines that are worth evaluating in field trials. The procedure is commonly referred to as Jenkins method B, the letter referring to one of the four methods of estimation originally evaluated by him.

The performance of a double-cross hybrid (P1 × P2) × (P3 × P4) is predicted by Jenkins method B from the formula

Double cross

$$(P1 \times P2) \times (P3 \times P4) = \tfrac{1}{4}[(P1 \times P3) + (P1 \times P4) + (P2 \times P3) + (P2 \times P4)]$$

where P1 × P3, P1 × P4, P2 × P3, and P2 × P4 are four of the six possible single crosses among the four lines. The two parental single crosses that would be involved in producing the double cross, P1 × P2 and P3 × P4, are not considered in making the prediction.

The principle of averaging nonparental single crosses to predict double-cross performance can be extended to the prediction of a three-way cross (P1 × P2) × P3.

$$\text{Three-way cross } (P1 \times P2) \times P3 = \tfrac{1}{2}[(P1 \times P3) + (P2 \times P3)]$$

To illustrate use of the formulas, assume that four inbred lines, A, B, C, and D, were evaluated in all single-cross combinations and the following yields were obtained in tons per hectare:

A × B = 8.8	B × C = 9.2
A × C = 8.9	B × D = 8.0
A × D = 8.4	C × D = 8.1

The predicted performance of the double cross (A × C) × (B × D) would be

$$\tfrac{1}{4}[(A \times B) + (A \times D) + (B \times C) + (C \times D)] = \tfrac{1}{4}[8.8 + 8.4 + 9.2 + 8.1] = 8.6$$

The predicted performance of the three-way cross (A × D) × C would be

$$\tfrac{1}{2}\,[(A \times C) + (C \times D)] = \tfrac{1}{2}\,[8.9 + 8.1] = 8.5$$

After a number of three-way or double-cross hybrids with high predicted performance are identified, the hybrids must be evaluated in field trials to determine which ones actually are superior. The prediction equations do not eliminate the need for field evaluation of three-way or double-cross hybrids, but they do reduce appreciably the number that are considered for testing.

SPECIAL CONSIDERATIONS WITH CYTOPLASMIC-GENETIC MALE STERILITY

When cytoplasmic-genetic male sterility is used for hybrid seed production, the procedures used to develop inbred lines for use as male parents may be different from those used to develop female parents.

Male Parents

The male parent of a hybrid must be male-fertile. For crop species whose commercial product is seed, the male parent also must possess genes that will result in a male-fertile hybrid. When a male-fertile hybrid is produced, the male parent is referred to as a restorer (R line), and the dominant nuclear genes that are responsible for hybrid fertility are known as restorer genes.

An inbred line being considered as an R line must be evaluated for its ability to produce a male-fertile hybrid (restoration ability) as well as for its combining ability. The tests for restoration and combining ability are made by crossing the potential R line to a tester that is male-sterile. The fertility of the testcross will determine the restoration ability of the line and the testcross yield will establish its combining ability. The evaluation for restoration and combining ability can be conducted at any level of inbreeding.

Female Parents

Female parents with cytoplasm that causes the pollen to be sterile and with recessive nonrestorer alleles that are unable to overcome the action of the cytoplasm are referred to as A lines. An A line cannot reproduce itself; therefore, an identical genotype with normal cytoplasm and nonrestorer alleles, known as a B or O line, must be developed first or concurrently with the A line.

The breeder begins with a segregating population of male-fertile plants that have normal cytoplasm and nonrestorer alleles. The population must be inbred, the combining ability of genotypes must be determined, and B line and A line versions of a superior inbred must be developed. In crops such as maize in which hybrid seed can be readily produced by artificial hybridization, a population can be inbred by self-pollination and the combining ability of male-fertile B lines can be determined by manually crossing them to R line testers. When a B line is found that would be useful in a commercial hybrid, the A line version can be developed by backcrossing. This option is not practical, however, for crops in which the quantities of testcross seed needed to evaluate combining ability cannot be produced artificially, such as sorghum.

For species in which testcross seed cannot be produced by artificial hybridization, a B line must be at least partially converted to its male-sterile (A line) counterpart to permit A line × R line testcrosses, or male sterility must be artificially induced in the B lines by chemical or physical means for seed production of B line × R line testcrosses.

When conversion of B lines to A lines is necessary to produce testcross seed, it is possible for inbreeding and selection of B lines per se, their conversion to cytoplasmic male sterility, and testing for general combining ability to be done concurrently. This can be illustrated with the development of inbred lines of sorghum. The following is a procedure for development of A and B lines of sorghum used by Fred Miller of Texas A & M University, College Station. In the procedure, all testcrosses and backcrosses involve the use of female plants that have cytoplasmic male sterility.

Season 1: F_2 plants are grown under disease conditions and desirable ones are selected. The plants are considered naturally self-pollinated, although a limited amount of outcrossing may occur.

Season 2: F_3 progeny of plants selected in season 1 are grown adjacent to a parent with cytoplasmic male sterility (cms). Desirable $F_{2:3}$ lines are selected, and selected plants in those rows are individually crossed to the cms parent. The selfed F_4 seed and F_1 seed of each F_3 plant are harvested.

Season 3: The cms-F_1 and F_4 progeny of each selected F_3 plant are grown in adjacent rows. (a) Three F_4 plants in each selected row are crossed to the adjacent cms-F_1 to obtain BC_1F_1 seed. The selfed F_5 seed and BC_1F_1 seed for each F_4 plant are harvested separately to continue inbreeding, selection, and backcrossing in season 4. (b) Two cms-F_1 plants are crossed to an R line tester and the testcross seed is used to evaluate the line for general combining ability in season 4. (c) Five F_1 plants are bagged to determine if they have any self-fertility. If an F_1 has self-fertility, the line is discarded because it would not be an acceptable maintainer line, that is, the progeny of the B line × A line cross would not be completely male-sterile as required for the female parent in hybrid seed production.

Season 4+: (a) The cms-BC_1F_1 and F_5 progeny of each selected F_4 plant are grown in adjacent rows. Three F_5 plants in each selected row are crossed to the adjacent cms-BC_1F_1 to obtain BC_2F_1 seed. The selfed F_6 seed and BC_2F_1 seed for each F_5 plant are harvested to continue inbreeding, selection, and backcrossing in season 5. (b) Two cms-BC_1F_1 plants are crossed to an R line tester different from the one used in season 3. The testcross seed is used to evaluate the line for general combining ability in season 5. (c) Five BC_1F_1 plants are bagged to evaluate self-fertility. (d) The cms-F_1 × R line testcrosses are evaluated for yield and other important traits in replicated tests. Lines that perform poorly are discarded, including backcross and inbred seed prepared in season 4.

The procedure used in season 4 is continued until the BC_5. Each season a different R line tester is used to evaluate general combining ability. Any superior female parents (BC_5 seed of the A line and F_9 seed of the corresponding B line) are released for use in commercial hybrid seed production.

IMPROVEMENT OF INBRED LINES BY BACKCROSSING

Backcrossing is widely used to convert B lines to A lines and to improve inbred lines by the transfer of genes for qualitative characters. A unique use of backcrossing is known as convergent improvement. Convergent improvement is a breeding method used to improve the performance of inbred lines that are parents of a hybrid cultivar. The unique aspect of the method is that the two inbred lines of a single cross are used as the donor parents for each other in a backcrossing and selection program.

During the early years of hybrid seed corn production, it was difficult to obtain inbred lines that had adequate productivity. To overcome the problem, Richey (1927) proposed the use of convergent improvement as a method of improving parent inbred lines without reducing the performance of the hybrid.

The procedure is illustrated here by assuming that four inbred parents are used to produce the double-cross hybrid (P1 × P2) × (P3 × P4). For convergent improvement, P1 and P2 would be the donor parents for each other and P3 and P4 would be reciprocal donor parents. Although the example involves improvement of P1 only, the four parents could be involved in four simultaneous backcrossing programs.

Season 1: P1 and P2 are crossed to obtain F_1 seed.

Season 2: F_1 plants of P1 × P2 are backcrossed to P1 to obtain BC_1F_1 seed.

Season 3: BC_1F_1 plants are grown and self-pollinated. Agronomically desirable BC_1F_1 plants are harvested individually.

Season 4: Progenies of the BC_1F_1 plants are grown in separate rows. The most desirable plants within the most desirable rows are backcrossed to P1 to obtain BC_2 F_1 seed. The BC_2F_1 seed from each BC_1F_2 plant is maintained separately.

Season 5: BC_2F_1 progenies of the BC_1F_2 plants are grown in separate rows. The most desirable plants within the most desirable rows are backcrossed to P1 to obtain BC_3F_1 seed. The BC_3F_1 seed from each BC_2F_1 plant is maintained separately. (For purpose of illustration, only three backcrosses will be used.)

Season 6: BC_3F_1 progenies of BC_2F_1 plants are grown in separate rows. The most desirable plants within the most desirable rows are self-pollinated. Seed from each selected BC_3F_1 plant is harvested separately.

Season 7: BC_3F_2 progenies of BC_3F_1 plants are grown in separate rows. The most desirable plants within the most desirable rows are self-pollinated. Seed from each selected BC_3F_2 plant is harvested separately.

Season 8: BC_3F_3 progenies of BC_3F_2 plants ($BC_3F_{2:3}$ lines) are grown in separate rows. The most desirable lines are self-pollinated and crossed to parent P2 to obtain P1* × P2 seed. P1* refers to the improved version of P1. Seed of the original P1 × P2 cross also is produced.

Season 9: P1* × P2 crosses involving different BC_3F_2-derived lines are compared with the original P1 × P2 cross. A BC_3F_2-derived line with testcross performance equal to or better than the original P1 × P2 cross is used to replace parent P1 in hybrid seed production.

The emphasis in convergent improvement is on the selection of dominant alleles, because they would be expressed in each backcrossing generation. Selection for recessive alleles would require progeny testing to identify the desired genotypes.

Several alterations can be made in the procedure just outlined. The number of backcrosses can be less or more than three, and selection for improved agronomic performance and uniformity may be practiced for more than two selfing generations.

PREPARATION OF BREEDER SEED

Breeder seed of an inbred line can be obtained by single-plant selection or progeny evaluation, and may involve self-pollination of sib mating. Because it is an integral part of hybrid seed production, the procedures for production of breeder seed are discussed in Chap. 35.

REFERENCES

Jenkins, M. T. 1934. Methods of estimating the performance of double crosses in corn. *J. Am. Soc. Agron.* 26:199–204.

Richey, F. D. 1927. The convergent improvement of selfed lines of corn. *Am. Nat.* 61:430–449.

Stadler, L. J. 1945. Gamete selection in corn breeding. *Maize Genet. Coop. Newsletter* 19:33–40.