CHAPTER TWENTY-TWO

Bulk Method

The bulk method is a procedure for inbreeding a segregating population until the desired level of homozygosity is achieved. The seed used to grow each inbreeding generation is a sample of that harvested from plants of the previous generation. The method is used primarily for development of self-pollinated cultivars, but could be used equally well to inbreed populations of cross-pollinated species.

The bulk breeding method was developed by Nilsson-Ehle at the Swedish Seed Association in Svalof, Sweden. The method, as used by the Association for development of wheat cultivars, was described by Newman (1912). Two parents were crossed and the segregating generations were grown en masse each generation. The object of this procedure was to allow the severe conditions of winter and early spring to either destroy or weaken plants with inadequate winter hardiness. Weak plants were manually eliminated from the population before harvest.

IMPLEMENTATION

The bulk method is characterized by its simplicity (Fig. 22-1):

Season 1: F_2 plants of a population are grown and their F_3 seeds are harvested in bulk.

Season 2: A sample of F_3 seeds from season 1 is planted, and F_4 seeds of the population are harvested in bulk.

The process is repeated until the desired level of inbreeding (homozygosity) is achieved, at which time individual plants are harvested from the population. The lines derived from the plants are evaluated for the characters of interest, in the same manner as lines derived by other methods of inbreeding.

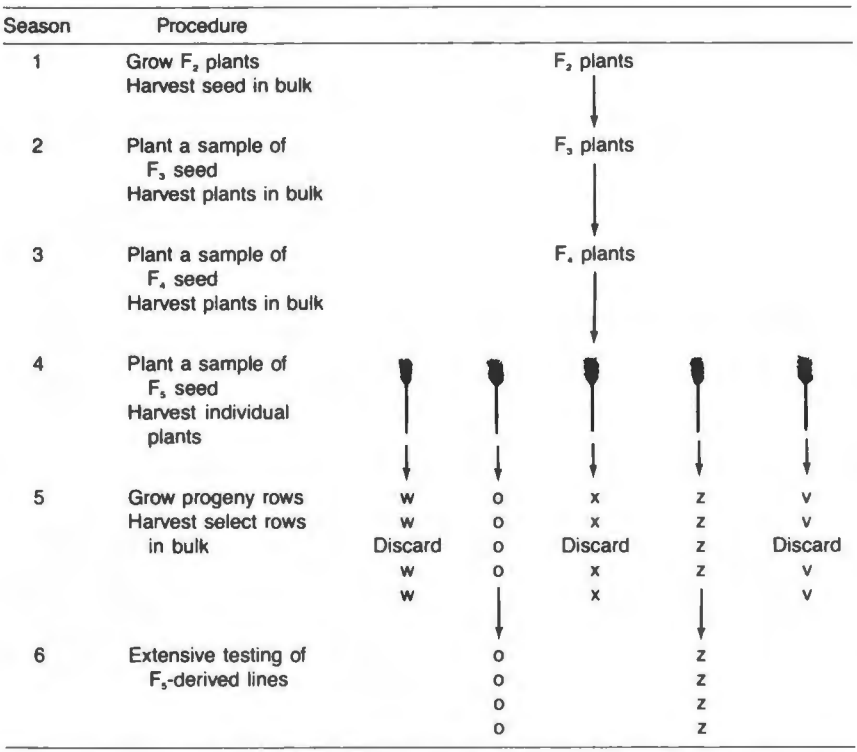


Figure 22-1 Illustration of the bulk method of inbreeding.

The influence of natural selection on the bulk method must be considered when selecting the environment in which to grow a population. If possible, a breeder chooses those environments in which natural selection is likely to favor the desired genotypes in the population. A population segregating for disease resistance would be grown in the presence of the pathogen, to reduce the productivity of susceptible plants or eliminate them from the population.

A primary concern with the bulk method is the avoidance of environments in which natural selection is likely to favor genotypes that are not considered desirable by the breeder. This concern has minimized use of the bulk method in greenhouses or off-season nurseries, in which the environment is considerably different from that encountered in the field where the population would normally be grown. Genotypes that have above-average productivity in the greenhouse or off-season nursery may have below-average productivity in the area for which a new cultivar is being developed.

The bulk method is commonly used in association with artificial mass selection (Ch. 24). Undesirable plants or parts thereof may be removed from a population and the remaining portion harvested in bulk. Special techniques may be used in sampling the most desirable seeds to plant the next generation.

GENETIC CONSIDERATIONS

The genotypic frequency in a population inbred by the bulk method is determined by the four variables associated with natural selection in a heterogeneous population: (a) genetic potential of a genotype for seed productivity, (b) competitive ability of a genotype, (c) influence of the environment on the expression of a genotype, and (d) sampling of genotypes to propagate the next generation. Because of these four variables, some of the plants in each segregating generation will not be represented by progeny in the next generation and some may be represented many times. There is no way to know if a particular F_2 plant has progeny represented in the F_3 or any later generation. There also is no way to predict the genetic variability for a character in any generation. If the four variables favor the desired genotypes, the frequency of these genotypes will be higher than in a population in which none of the variables favor them. The amount of increase in the frequency of the desired genotypes is difficult to predict, because the influence of the four variables may not be consistent from one generation to the next.

Suneson (1949) demonstrated that natural selection does not always have positive effects on a population. When he grew a mixture of four similarly adapted barley cultivars in bulk for 16 years, two of the component cultivars were practically eliminated. One of the two had a significantly better yield and leaf disease resistance than any of the others when grown in pure stands. The cultivar that predominated in the mixture had the poorest leaf disease resistance and a mean yield below the median for the component cultivars.

MERITS OF THE BULK METHOD

The bulk method has both advantages and disadvantages.

Advantages

1. The bulk method is an easy way to maintain populations during inbreeding.
2. Natural selection is permitted to occur, which can increase the frequency of desired genotypes compared with an unselected population.
3. The bulk method can be used readily in association with mass selection with self-pollination.

Disadvantages

1. Plants of one generation are not all represented by progeny in the next generation.

2. Genotypic frequencies and genetic variability in the population cannot be defined readily.
3. The bulk method is not suited to greenhouses and off-season nurseries if the performance of genotypes is not representative of the area in which the genotypes normally are grown.
4. Natural selection may favor undesirable rather than desirable genotypes.

REFERENCES

Newman, L. H. 1912. *Plant breeding in Scandinavia*. Canadian Seed Grower's Association, Ottawa.

Suneson, C. A. 1949. Survival of four barley varieties in a mixture. *Agron. J.* 41:459–461.

CHAPTER TWENTY-THREE

Single-Seed Descent Method

Single-seed descent is a method of inbreeding a segregating population that is especially well suited to environments that are not representative of those in which the segregates would be grown commercially. The method can be used readily in both self-pollinated and cross-pollinated species.

The single-seed descent method resulted from the interest of plant breeders in rapidly inbreeding populations before beginning the evaluation of individual lines. The concept of rapid inbreeding before selection was proposed by Goulden (1941). He noted that a wheat breeding program could be divided into two aspects: the development of pure lines from a segregating population and selection among the pure lines for those with the desired characteristics. He indicated that with the pedigree method of inbreeding, single plants had to be grown in environments in which genetic differences would be expressed for the characters under selection. This meant that only one generation could be grown each year. He suggested as an alternative that inbreeding and selection be separated in a breeding program. He proposed that the number of progeny grown from a plant each generation be reduced to a minimum of one or two, and that two generations be grown in the greenhouse during the winter and one in the field during the summer. In this manner, the F_6 generation could be attained in 2 years. With pedigree selection at least 5 years are needed to reach the F_6 generation. After the desired level of homozygosity was achieved, a large number of lines could be tested for the desirable characters. The term single-seed descent was not used by Goulden to describe his procedure, but the essential aspects of the method are included in his proposed system of inbreeding.

The concept of inbreeding a large number of lines to homozygosity before selection for yield was described by Jones and Singleton (1934). They described a procedure that provided inbred lines that traced to different individuals in the initial segregating population. They grew the progeny of individual plants by

removing seeds from an ear, wrapping them in tissue paper, and planting the wrapped seeds in a hill the next generation. The hills were thinned to three plants, two of the plants were self-pollinated, and one of the two was harvested to continue inbreeding. No labeling of individual lines was done during inbreeding. They indicated that the procedure made it possible to produce a large number of inbred lines in a small area with a minimum of labor for hand-pollination.

The harvest of single seed from plants during inbreeding was described by Johnson and Bernard (1962) for soybeans. In their publication, the term single-seed descent was used for the first time in the literature. The term seems to have been the result of discussions with C. A. Brim, who first used the procedure in soybeans (H. W. Johnson, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, personal communication). The method of single-seed descent later was described by Brim (1966), who referred to it as a modified pedigree method.

ALTERNATIVE PROCEDURES

Plant breeders use three procedures to implement the concept of single-seed descent.

Single-Seed Procedure

The classic procedure of single-seed descent is to harvest a single seed from each plant in a population, bulk the individual seeds, and plant the entire sample the next generation (Fig. 23-1):

Season 1: F_2 plants of a population are grown. One F_3 seed per plant is harvested from all plants, and the seeds are bulked. A separate reserve sample of one seed per plant is harvested from the population.

Season 2: The bulk of F_3 seed from season 1 is planted. One F_4 seed per plant is harvested from all plants, and the seeds are bulked. A separate reserve sample of one seed per plant is harvested from the population.

The procedure is repeated until the desired level of inbreeding (homozygosity) is achieved. Individual plants are harvested and the lines derived from them are evaluated for the characters of interest.

When the single-seed procedure is used, the size of the population will decrease each generation because of lack of seed germination or failure of plants to produce seed. It is necessary to decide on the number of inbred plants that are desired the last generation and begin with an appropriate population size in the F_2 generation. For example, assume that 200 F_4 plants are desired from a segregating population. Assume that 70 percent of the seeds planted will produce a plant with at least one seed. To calculate the number of seeds needed each generation, it is necessary to begin with the F_4 generation and work backward to the F_2 generation.

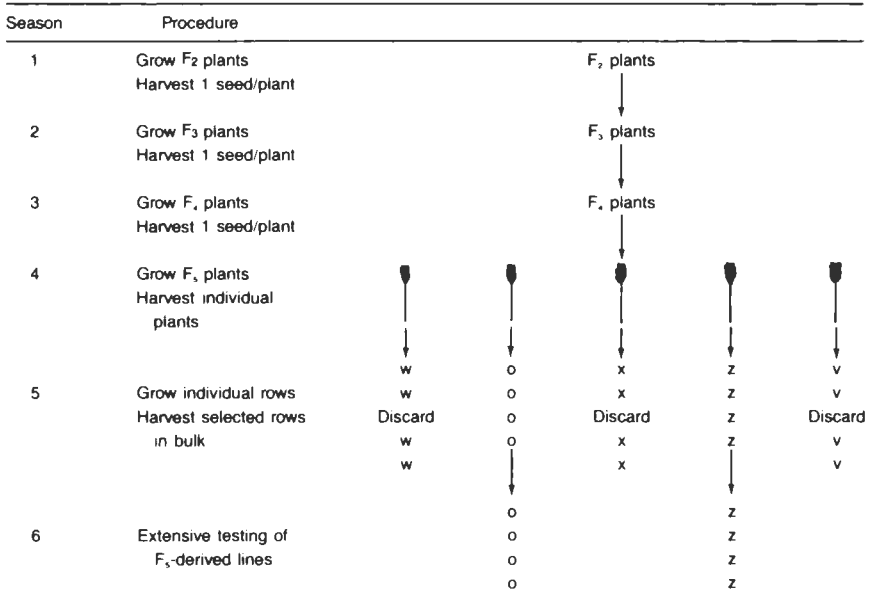


Figure 23-1 Illustration of the single-seed procedure for the single-seed descent method. (Courtesy of Fehr, 1978.)

F_4: Plant 286 F_4 seeds to obtain 200 F_4 plants (200/0.7 = 286).
F_3: Plant 409 F_3 seeds to obtain 286 F_3 plants and 286 F_4 seeds (286/0.7 = 409).
F_2: Plant 584 F_2 seeds to obtain 409 F_2 plants and 409 F_3 seeds (409/0.7 = 584).

A reserve sample of seed can be harvested at the same time as the sample for planting, or the reserve sample can be harvested as a separate operation. Brim (1966) suggested harvesting a two- or three-seeded pod of soybeans and using one of the seeds for planting and the remaining seeds for a reserve.

The single-seed procedure ensures that each individual in the final population traces to a different F_2 individual. However, the procedure cannot ensure that a particular F_2 will be represented in the final population, because failure of any seed to germinate automatically eliminates that seed's F_2 family.

Single-Hill Procedure

The single-hill procedure can be used to ensure that each F_2 plant will have progeny represented in each generation of inbreeding. It is based on the technique described by Jones and Singleton (1934) (Fig. 23-2). Progeny from individual plants are maintained as separate lines during each generation of inbreeding by

Season	Procedure					
1	Grow F_2 plants Harvest F_3 seed from each plant individually					
2	Grow individual hills Harvest F_4 seed from each hill individually	w w w w	o o o o	x x x x	z z z z	v v v v
3	Grow individual hills Harvest F_5 seed from each hill individually	w w w w	o o o o	x x x x	z z z z	v v v v
4	Grow individual hills Harvest one F_5 plant from each hill	w w w w	o o o o	x x x x	z z z z	v v v v
5	Grow individual rows Harvest selected rows in bulk	w w w w	o o Discard o o	x x Discard x x	z z z z	v v v v
6	Extensive testing of F_5-derived lines	w w w w			z z z z	v v v v

Figure 23-2 Illustration of the single-hill procedure for the single-seed descent method. (Courtesy of Fehr, 1978.)

planting a few seeds in a hill, harvesting self-pollinated seeds from the hill, and planting them in another hill the following generation. An individual plant is harvested from each line when the population has reached the desired level of homozygosity.

Season 1: F_2 plants of a population are grown, and F_3 seeds are harvested from each. Part of the seed from each plant is used in season 2 and part is kept as a reserve.

Season 2: A hill is grown for each $F_{2:3}$ line, and F_4 seeds are harvested from the hill. Part of the seed is used in season 3 and part is kept as a reserve.

The procedure is repeated until the desired level of inbreeding is achieved, at which time individual plants are harvested.

With the single-hill procedure, the identity of each F_2 plant and its progeny can be maintained during self-pollination. When the identity of an F_2 is maintained, the seed packet and hill must be properly identified with a line designation for planting and harvest.

Multiple-Seed Procedure

Use of the single-seed procedure requires that the size of the population in F_2 be larger than in later generations, due to lack of seed germination, and that two samples be harvested, one for planting the next generation and one for a reserve. To avoid these problems, breeders sometimes bulk two or three seeds from each plant during harvest. Part of the sample is planted and the remaining part is used as a reserve. The procedure will be referred to as the multiple-seed procedure, in contrast to the single-seed procedure. Some breeders refer to the procedure as modified single-seed descent, and others describe it by the method of harvest. For example, soybean breeders who harvest a bulk of one pod per plant, each containing several seeds, sometimes refer to it as the pod-bulk method. Steps in the multiple-seed procedure are as follows:

Season 1: F_2 plants of a population are grown. A similar number of F_3 seeds, usually two to four, is harvested from all plants, and the seeds are bulked. Part of the seed is used in season 2 and part is kept as a reserve.

Season 2: A sample of F_3 seed from season 1 is planted. A similar number of F_4 seeds is harvested from all plants and the seeds are bulked. Part of the seed is used in season 3 and part is kept as a reserve.

The procedure is repeated until the desired level of inbreeding is achieved, at which time individual plants are harvested.

The number of seeds planted and harvested each season depends on the number of lines desired from the population and the anticipated germination percentage of the seed. With the multiple-seed procedure, the number of seeds planted can be constant each season. Assume that the breeder desires 200 F_4 plants from a segregating population and that the survival rate is 70 percent each generation. The procedure would be as follows.

F_2: Plant 286 F_2 seed to obtain 200 F_2 plants that will produce seed (200 plants/0.7 = 286 seeds). Harvest three seeds from the 200 plants for a total of 600 F_3 seeds.

F_3: Plant 286 F_3 seeds and put the remaining 314 seeds in reserve. Harvest three seeds from the 200 surviving plants for a total of 600 F_4 seeds.

F_4: Plant 286 F_4 seeds and put the remaining 314 seeds in reserve. Harvest 200 F_4 plants.

RAPID GENERATION ADVANCE

Single-seed descent is well suited for use in greenhouses and winter nurseries where genotypes perform differently than in their area of adaptation. This approach is particularly useful when breeders modify the environment to reduce

the time required to produce a generation of seed. Major (1980) reviewed the principles involved in rapid seed production.

> Rapid production of hybrid and self-pollinated seed can reduce the length of time required to develop a new cultivar. However, shortening the life cycle by manipulation of the environment will reduce seed yield per plant. It also may reduce flower size and increase cleistogamy, thereby limiting such techniques to the production of self-pollinated seed.
>
> Dormancy and vernalization are survival mechanisms common to some species, and these can be overcome artificially. Seed of species that are difficult to germinate because of a hard seed coat should be scarified before seeding. Other types may require more specialized treatments to overcome dormancy. Wild rice must have the pericarp removed from around the embryo, hop needs a chilling period of 5 to 8 weeks, and some rye cultivars need an afterripening period of about 20 days. In some cases, the afterripening period can be overcome chemically with potassium nitrate or gibberellic acid (Spicer and Dionne, 1961).
>
> Treatment of germinating seeds or seedlings in growth chambers at temperatures of 2 to 5°C will substitute for natural vernalization or overcome the biennial nature of a species. In winter wheat, exposure to 2 to 5°C in short days for 8 weeks is the most effective way of meeting the vernalization requirement (Grant, 1964). Sugarbeet is a biennial and the shortest life cycle occurs when it is exposed to 4 to 7°C for about 10 weeks during floral induction (Gaskill, 1952).
>
> Continuous light is the most suitable method of reducing the time to flowering of long-day plants. Short-day lengths promote development of short-day plants, but the actual day length used will depend on the optimum photoperiod. For plants of tropical origin, an 8-hour day may be most suitable. The use of an 8-hour day in Puerto Rico reduced the days from planting to flowering of pigeonpea by 4 months in medium maturity cultivars and 7 weeks in late cultivars (Sharma and Green, 1980). A 12-hour day may be adequate for crops adapted to more temperate regions.
>
> The temperatures most suitable for rapid seed production generally vary from about 25 to 35°C, depending on whether it is a cool or warm-season crop. Some species, such as pigeonpea or pearl millet, will develop most rapidly at temperatures above 35°C.
>
> Moisture stress may be used to reduce the time taken from flowering to maturity, but stress should not be used until the developing seeds are well established and will not abort.
>
> Soil fertility should be limited to the amount needed to produce a small plant with a few seeds. Nitrogen stress during the seed-filling period will result in smaller, earlier-maturing seeds. Phosphorus tends to have the opposite effect to N, so a liberal application of P may also hasten maturity.
>
> High population densities often appear to hasten maturity, but this is probably the result of fertility and moisture stresses that occur late in the seed-filling period. With excess fertility and moisture, increased population density may actually delay maturity.
>
> Most crops harvested for their seed have been selected for large seed. The embryos of such seed may reach maturity long before maximum seed dry weight is reached. Thus it may be possible to harvest seed 10 to 15 days after the linear phase of seed filling has begun. The seed may be small and shrivelled, but may still have excellent viability. When this procedure is followed, it is advisable to cut the entire plant and

dry it in the field or the greenhouse. This method essentially imposes severe moisture stress, and curing the whole plant allows some translocation of sugars and minerals into the seed during the drying period. [Major, 1980]

Selection on a single-plant basis can be practiced during any generation of single-seed descent (Brim, 1966; Jones and Singleton, 1934). In greenhouse and winter nurseries, the expression of certain characters may be limited and selection may be ineffective. However, seed weight in soybean is an example of a character for which selection can be practiced effectively in a winter nursery (Bravo et al., 1980).

GENETIC CONSIDERATIONS

Genetic expectations for populations maintained without artificial selection during inbreeding by the single-seed, single-hill, or multiple-seed procedures are those of an idealized diploid population. The frequency of heterozygous individuals for a single locus is 1/2 in F_2 and decreases by 50 percent each generation of inbreeding. The additive genetic variability among individuals in the population increases at the rate of $(1 + F)\,\sigma_A^2$, where F is the inbreeding coefficient. F is 0 in F_2, 1/2 in F_3, 3/4 in F_4, and so forth.

There is no natural selection in populations maintained by single-seed descent, unless genotypes differ in seed germination potential or the environment prevents some genotypes from setting any seed. The genetic potential of a genotype for seed productivity or its competitive ability does not influence single-seed descent, because a similar number of seed is harvested from each plant, regardless if the plant has 3 or 3000 seeds.

The multiple-seed procedure is subject to variation associated with sampling of seed from a bulk sample to plant the next generation. The sampling results in the exclusion of progeny from some plants and the multiple representation of progeny from others. Genetic variability in the population may be decreased whenever two or more individuals trace to the same F_2 plant, instead of every individual tracing to a different F_2.

MERITS OF THE SINGLE-SEED DESCENT PROCEDURES

Single-Seed, Single-Hill, and Multiple-Seed Procedures Collectively

Advantages.

1. They are an easy way to maintain populations during inbreeding.
2. Natural selection does not influence the population, unless genotypes differ in their ability to produce at least one viable seed each generation.
3. The procedures are well suited to greenhouse and off-season nurseries where the performance of genotypes may not be representative of their performance in the area in which they normally are grown.

Disadvantages.

1. Artificial selection is based on the phenotype of individual plants, not on progeny performance.
2. Natural selection cannot influence the population in a positive manner, unless undesirable genotypes do not germinate or set any seed.

Single-Seed Procedure

Advantages.

1. This procedure requires considerably less time and land area than the single-hill procedure.
2. Every plant in the population traces to a different F_2, which results in greater genetic variability in the population.

Disadvantages.

1. Every F_2 plant may not be represented by a progeny in the population due to the failure of some individuals to produce at least one viable seed each generation of inbreeding.
2. The size of the population for the single-seed procedure must be adjusted for germination percentage.
3. The procedure requires more time than the multiple-seed procedure at harvest to obtain one sample for planting the next generation and one to keep as a reserve.

Single-Hill Procedure

Advantage. Every plant in the population traces to a different F_2, which results in greater genetic variability in the population.

Disadvantages.

1. It requires more time at planting and harvest than the other two procedures.
2. It requires more land area than the other two procedures.

REFERENCES

Bravo, J. A., W. R. Fehr, and S. Rodriquez de Cianzio. 1980. Use of pod width for indirect selection of seed weight in soybeans. *Crop Sci.* 20:507–510.

Brim, C. A. 1966. A modified pedigree method of selection in soybeans. *Crop Sci.* 6:220.

Fehr, W. R. 1978. Breeding. pp. 120–155. *In* A. G. Norman (ed.), *Soybean physiology, agronomy, and utilization*. Academic Press, New York.

Gaskill, J. O. 1952. A new sugarbeet breeding tool—two seed generations in one year. *Agron. J.* 44:338.

Goulden, C. H. 1941. Problems in plant selection. pp. 132–133. In *Proceedings of the 7th International Genetical Congress*, Edinburgh.

Grant, M. N. 1964. Vernalization and days to anthesis of winter wheat under controlled temperature and light. *Can. J. Plt. Sci.* 44:446–450.

Johnson, H. W., and R. L. Bernard. 1962. Soybean genetics and breeding. *Adv. Agron.* 14:149–221.

Jones, D. F., and W. R. Singleton. 1934. Crossed sweet corn. Conn. Agr. Exp. Sta. Bull. 361:487–536.

Major, D. J. 1980. Environmental effects on flowering. pp. 1–15. *In* W. R. Fehr and H. H. Hadley (eds.), *Hybridization of crop plants*. American Society of Agronomy, Madison, Wis.

Sharma, D., and J. M. Green. 1980. Pigeonpea. pp. 471–481. *In* W. R. Fehr and H. H. Hadley (eds.), *Hybridization of crop plants*. American Society of Agronomy, Madison, Wis.

Spicer, P. B., and L. A. Dionne. 1961. Use of gibberellin to hasten germination of *Solanum* seed. *Nature* 189:327–328.

CHAPTER TWENTY-FOUR

Mass Selection in Self-Pollinated Populations

Mass selection in a heterogeneous population is the oldest method of crop improvement in self-pollinated species. Before modern plant breeding began, farmers selected desirable plants or seeds from heterogeneous native populations to plant the next crop. Differences in the use of a crop and in personal preferences for appearance led farmers to develop by mass selection a wide diversity of cultivars, often referred to as landraces. Mass selection later was adopted by plant breeders as a method of increasing the frequency of desirable genotypes during inbreeding in populations developed by hybridization or artificial mutagenesis.

In the early years of plant breeding, mass selection was the primary method used to improve a crop. Individuals were selected from a heterogeneous cultivar or from a segregating population developed by artificial hybridization, the selected individuals were threshed together, and the bulk sample was planted the next generation (Newman, 1912).

It is important to realize that in the early years of plant breeding, the genetic principles developed by Mendel and the pure-line theory of selection identified by Johannsen were not available. The breeders selected heterogeneous cultivars for various characters without knowing the genetic makeup of the material or the genetic consequences of their activity.

IMPLEMENTATION

Cultivar Development

Mass selection with self-pollination has two aspects: (a) selection of individuals and (b) sampling of seed from the selected individuals to plant the next gener-

ation. Selection and sampling may be done simultaneously or may be distinct operations.

Selection. The following examples demonstrate the diversity of selection strategies that breeders use.

1. *Mass selection for plant height in an oat population (Romero and Frey, 1966)*: A population of plants was grown in the field. After flowering was completed, plants were trimmed with a lawn clippers to the height of the panicle tips of 'Cherokee,' a cultivar of desirable height. Trimming removed the entire panicle of excessively tall plants, partially removed the panicles of other plants, and did not affect the panicles of short plants. To eliminate excessively short plants, only the top 10 cm of the trimmed plants was harvested at maturity, dried, and threshed in bulk. There was about 23 kg of seed in the bulk. A 3-kg sample was used to plant the next generation, a 1-kg sample was placed in cold storage, and the remaining seed was discarded.
2. *Mass selection for maturity, seed size, and seed composition in a soybean population (Fehr and Weber, 1968)*: A population of plants was grown in the field, and 400 plants of early maturity were selected. The top one-fourth of the main stem and branches of all plants was cut off to remove poorly developed seed, then the remainder of the plants was threshed together in bulk. The seed bulk was passed over a series of sieves with slots of different widths, and the 25 percent of the seeds with the largest size was retained. This large-seed fraction was passed through a series of glycerol–water solutions of differing specific gravities in which seeds with higher density sank and those with lower density floated. The 25 percent of the seeds that sank because of their high density were washed in water, air-dried, and used for planting the next generation.
3. *Mass selection for tolerance to calcareous soils and maturity in a soybean population (W. R. Fehr, unpublished data)*: A population of plants was grown on calcareous soil in the field, where yellowing is expressed by genotypes that lack the ability to utilize available iron in the soil. Cultivars with desirable levels of tolerance to calcareous soils and the desired maturity were planted as standards for selection. Plants with more yellowing than expressed by the standards were removed before flowering began. Plants earlier or later in maturity than the standards also were discarded. One pod per plant was harvested and bulked from selected individuals. Part of the seed bulk was used to plant the next generation and the other part was retained as a reserve.

Selection has been applied to individual plants or seeds. It has involved artificial selection, natural selection, or both. Selection has been practiced for one or for multiple characters in a population. The procedures used for selection generally are rapid and inexpensive to apply.

Sampling. Selected plants or parts of plants often are threshed in bulk, and a random sample of seeds from the bulk is planted the next generation (refer to the Romero and Frey example). Alternatively, an equal quantity of seeds harvested from selected plants may be used for the next generation (see the Fehr example). When selection is conducted on seeds, the selected sample may be planted the next generation; therefore, selection and sampling are done simultaneously (Fehr and Weber example).

Cultivar Purification

Mass selection is used in the maintenance of purity for self-pollinated cultivars. It may involve roguing off-type plants from a field or the elimination of off-type seeds in a harvested sample. The use of mass selection for cultivar purification is discussed in Chap. 31.

GENETIC CONSIDERATIONS

Effective mass selection in a self-pollinated population will result in a higher percentage of desirable genotypes than would be possible if no selection were practiced during inbreeding. The effectiveness of selection is a function of the heritability of the character on a plant or seed basis in the environment in which the population is grown. Anything that can be done to enhance genetic differences among genotypes or to reduce environmental variation will increase heritability and the effectiveness of mass selection. For example, mass selection for disease resistance will be most effective when a uniform infestation of the pathogen is present.

The genetic consequence of mass selection in a self-pollinated population is markedly different from that of mass selection in a cross-pollinated population. In a self-pollinated population, the frequency of heterozygous individuals declines progressively with each generation of inbreeding, and the frequency of homozygous individuals increases, regardless of the effectiveness of selection. In a cross-pollinated population, the frequency of heterozygous individuals is a function of allelic frequency. The relative proportion of homozygous and heterozygous individuals in a cross-pollinated population will not change unless selection is effective in altering the frequency of alleles controlling the character of interest.

It would be best if the term mass selection was used either for self-pollinated populations or for cross-pollinated populations, but not for both. Breeders are not likely to abandon the term, however, because it has been used for so long in both types of populations. It is important, therefore, that a speaker or writer describe as clearly as possible whether mass selection is accompanied by self-pollination or by cross-pollination. Use of the term mass selection with self-

pollination or mass selection with cross-pollination would provide the necessary clarification. Because breeders do not always adequately describe their use of mass selection, the listener or reader must determine which mode of reproduction is present in the mass selection program being described. The situation is particularly confusing for self-pollinated species when male sterility is available to permit cross-pollination among individuals in a population. With genetic male sterility, a breeder could use mass selection with cross-pollination and mass selection with self-pollination in a single population.

In this chapter, mass selection has been discussed as it is used in self-pollinated populations. Mass selection for cross-pollinated populations is discussed in Chaps. 15 and 16.

MERITS OF MASS SELECTION

Advantage

Mass selection with self-pollination can be a rapid and inexpensive procedure for increasing the frequency of desired genotypes in a population during inbreeding.

Disadvantages

1. Mass selection can only be used in environments in which the character is expressed. This may prevent its use in off-season nurseries or greenhouses, where expression of the character may be absent or altered.
2. The effectiveness of mass selection depends on the heritability of the character on a plant or seed basis. Mass selection is of limited value for characters of low heritability.

REFERENCES

Fehr, W. R., and C. R. Weber. 1968. Mass selection by seed size and specific gravity in soybean populations. *Crop Sci.* 8:551–554.

Newman, L. H. 1912. *Plant breeding in Scandinavia*. Canadian Seed Grower's Association, Ottawa.

Romero, G. E., and K. J. Frey. 1966. Mass selection for plant height in oat populations. *Crop Sci.* 6:283:287.

CHAPTER TWENTY-FIVE

Pedigree Method

The pedigree method is used during the inbreeding of populations of self- and cross-pollinated species for the development of desirable homogeneous lines. There is no difference in its implementation for self- or cross-pollinated species, except that inbreeding by self-pollination occurs naturally in the former and must be done manually in the latter.

The term pedigree selection was first employed when single-plant selection was used to isolate pure lines in heterogeneous landraces of self-pollinated species. Newman (1912) described how pedigree selection was discovered at the Swedish Seed Association in Svalof, Sweden. He indicated that until 1891, mass selection with self-pollination was used to improve cultivars of self-pollinated species. Desirable plants were selected from an existing cultivar or segregating population and threshed together in bulk to propagate the next generation. During the harvest of 1891, Hjalmar Nilsson collected seed from individual plants of wheat and other species. Each was classified botanically and morphologically, plants of a similar class were threshed together, and a sample was planted in a row the next season. Some classes had only one individual plant to represent them, and the seed from the plant was sown by itself in a row the next generation. Detailed plant observations were made on the progeny rows. During examination of the data it was noted that only the plants in those rows that represented the progeny of a single plant had uniform characteristics. This observation indicated that the most effective way to obtain a uniform genotype was to grow the progeny of a single plant. The use of single-plant selection and progeny evaluation was referred to by Nilsson as the "system of pedigree." It should be noted that the pedigree method also was developed independently by Vilmorin of France and was designated the "Vilmorin system of selection."

By the time inbreeding of cross-pollinated species became important, the value of pedigree selection in self-pollinated species was well documented. Breeders of cross-pollinated crops adopted the method readily.

IMPLEMENTATION

Pedigree selection generally begins with an F_2 or S_0 population and continues until homogeneous lines are developed. A general outline of the pedigree method is provided in the following description and in Fig. 25-1. Selection first is practiced among F_2 plants. In the next season, the most desirable $F_{2:3}$ lines are chosen, then desirable F_3 plants are selected from within the selected lines. The following season and in all subsequent generations of inbreeding, the most desirable families are identified first, then desirable lines within the selected families are chosen, and finally desirable plants within selected lines are harvested individually. A family refers to lines that were derived from plants selected from the same progeny row the preceding generation.

Season 1: The F_2 (S_0) population is space planted to permit individual plant selection. Enough F_2 plants are self-pollinated to ensure that an adequate number of desirable ones will be available. Desirable F_2 plants are selected and maintained separately.

Season 2: The F_3 progeny of each selected F_2 plant ($F_{2:3}$ line) are grown in a row. Adequate space is left between individuals for single-plant selection. Enough plants are selfed within each row to ensure that some selection can be practiced among plants, if the row is desirable. The best rows are selected; then the best selfed plants within those rows are chosen.

Season 3: The F_4 progeny of each selected F_3 plant ($F_{3:4}$ line) are grown in a row. The progeny of plants that came from the same row in season 2 are grown as a family in adjacent rows. Plants within each row are selfed as in season 2. The best families are selected, then the best row(s) within selected families, and then the best selfed plants within the best rows.

The procedure of season 3 is repeated until homogeneous lines are identified. The homogeneous lines are harvested individually in bulk, and evaluated in replicated tests during subsequent seasons.

Number of Selections

One consideration with the pedigree method is the number of plants, lines, or families to select each generation. A number of factors are included in making the decision.

Available Resources. In any breeding program, the number of genotypes evaluated depends on the resources available, including time, personnel, land, and so forth. For the pedigree method, the breeder should establish the total number of progeny rows that can be grown and the number of individual plants that can be evaluated each season for all generations of selection. The outline of the pedigree method described above and in Fig. 25-1 provides the stepwise procedure that would be used for a new population. In an established breeding

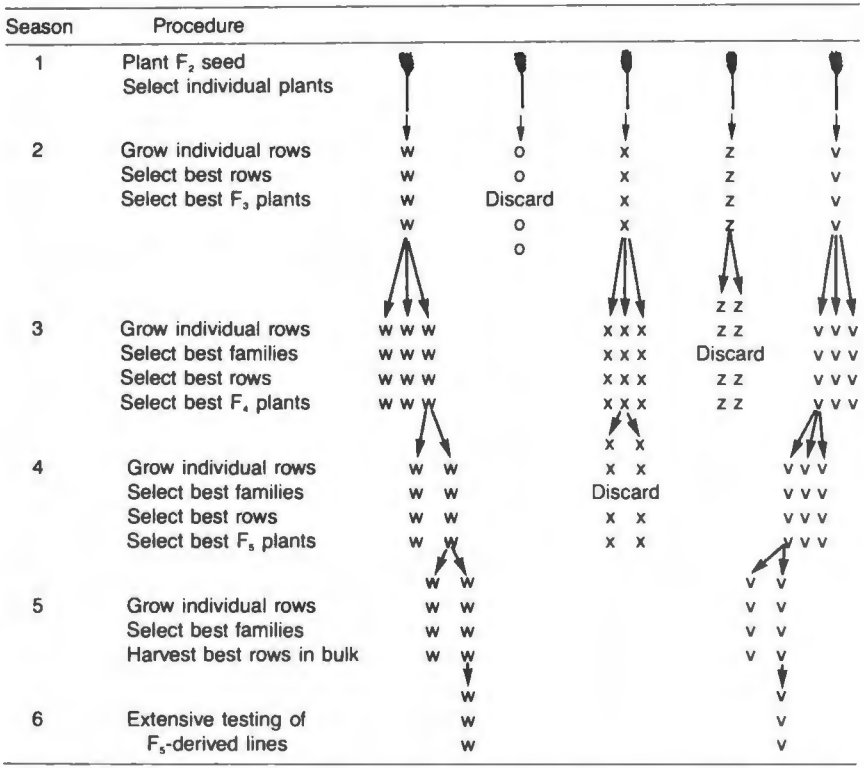


Figure 25-1 Illustration of the pedigree method. (Courtesy of Fehr, 1978.)

program, however, every step of the procedure is carried out the same season with breeding material that entered the program in different years. In any season, a breeder would have in the field F_2 plants, $F_{2:3}$ lines, $F_{3:4}$ lines, $F_{4:5}$ lines, and so forth. For that reason, total resources available for a program of pedigree selection must be considered.

Expected Genetic Variability. The genetic variability expected each generation influences the number of selections made (Table 25-1). The additive genetic variability among $F_{2:3}$ lines is greater than the variability that will be expressed within lines during the first few generations of inbreeding. It is important, therefore, to maximize the number of $F_{2:3}$ lines that are evaluated and to retain as many different F_2 families as possible for replicated testing after pedigree selection is completed. One reason the ancestry of each line is kept during pedigree selection is to minimize the relationship among lines selected in the program, with special emphasis on the number of F_2 families retained.

When plant selections are made within lines for each generation of inbreeding,

Table 25-1 Additive Genetic Variability Expected Among and Within Lines During Inbreeding without Natural or Artificial Selection

Generation of Lines	Additive Genetic Variability	
	Among Lines	Within Lines
$F_{2:3} = S_{0:1}$	1*	$\frac{1}{2}$
$F_{3:4} = S_{1:2}$	$1\frac{1}{2}$	$\frac{1}{4}$
$F_{4:5} = S_{2:3}$	$1\frac{3}{4}$	$\frac{1}{8}$
$F_{5:6} = S_{3:4}$	$1\frac{7}{8}$	$\frac{1}{16}$

*The additive genetic variability among F_2 or S_0 plants is considered to be 1, the same as the variability among $F_{2:3}$ or $S_{0:1}$ lines.

the additive genetic variability expressed within lines is reduced to half of that present in the preceding generation (Table 25-1). As a result, the number of plants selected within lines generally decreases with each generation of inbreeding. For example, a breeder may choose up to four plants within desirable $F_{2:3}$ lines but not more than two in an $F_{4:5}$ line.

Number of Generations of Selection. The number of generations that pedigree selection is conducted will influence the amount of resources that can be allocated to each generation. As the genetic variability within lines decreases, the uniformity of lines increases (Table 25-1). The amount of genetic variability expressed within a group of lines is not the same; therefore, $F_{2:3}$ lines may be as uniform for visual characters as are $F_{5:6}$ lines, although the percentage of uniform $F_{2:3}$ lines will be less. Pedigree selection can be discontinued for a line whenever an adequate degree of uniformity is achieved, so no generation is specified as the one when a line will be harvested in bulk to initiate replicated testing. However, most breeders would choose a generation to terminate pedigree selection and would discard all heterogeneous lines that still remain.

Characters for Selection

Effective pedigree selection can be carried out only for characters with an adequate heritability for individual plants, progeny rows, or both. Pedigree selection usually is associated with visual selection, but it can be used equally well with characters that cannot be seen, such as protein composition of seed. For characters that do not involve visual selection, the amount of error associated with measurement of the character influences its heritability. When visual selection is involved, the ability of an individual to consistently discern true genetic differences is an important component of experimental error and heritability.

The relationship between genetic improvement and number of characters under selection also is an important factor to consider with pedigree selection.

Assuming that no linkage is present between genes, the frequency of lines with two or more desirable characters is determined by multiplying together the independent frequencies for each character. If the frequency of desirable lines for standability is 1/10, plant color 1/4, leaf type 1/8, disease resistance 1/4, and fruiting habit 1/6, the frequency of lines that are desirable for all characters is 1/7680. Therefore, the number of characters under selection influences the number of plants and lines that must be grown. It also influences the amount of genetic variability that will be available for characters to be evaluated in replicated tests after pedigree selection is completed, such as for seed yield.

Environments for Selection

The expression of genetic differences among plants or lines requires appropriate environmental conditions. There is no value in selection for standability if all plants or lines are erect due to an environment with insufficient moisture, or in selection for disease resistance if the pathogen is not present. Pedigree selection can only be effective in environments where genetic differences are expressed, which often prevents the use of the method when greenhouses or off-season nurseries are used. Breeders interested in rapid inbreeding through the use of off-season facilities, where characters of interest are not expressed, must use a method other than pedigree selection or use it in combination with other methods.

Record Keeping

Records are more extensive for pedigree selection than for any other method of inbreeding. A population designation usually is followed by a number for the plant selected each generation. The numbering begins with the first generation in which the plants are genetically different. There is no need to identify F_1 plants of a population unless they are considered genetically unique. For purposes of illustration, we will follow the record for a single-cross population.

F_2: Assume 1000 F_2 plants are grown and 100 are selected from population AX1214. The 100 plants would be labeled with the population designation and a number designating the F_2 plant selected: AX1214-1, AX1214-2, and AX1214-3.

F_3: Progeny from each F_2 plant are grown in a row. Assume that three plants are chosen from rows AX1214-2 and AX1214-11. The plants would be labeled with the population designation, the F_2 plant number, and a number for the F_3 plant selected: AX1214-2-1, AX1214-2-2, and AX1214-2-3; AX1214-11-1, AX1214-11-2, and AX1214-11-3.

F_4: Progeny from each F_3 plant are grown in a row. Assume that two plants are chosen from rows AX1214-2-2 and AX1214-11-3. The plants would be labeled with the population designation, the F_2 and F_3 plant

numbers, and a number for the F_4 plant selected: AX1214-2-2-1 and AX1214-2-2-2; AX1214-11-3-1 and AX1214-11-3-2.

F_5: Progeny from each F_4 plant are grown in a row. Assume that rows AX1214-2-2-1 and AX1214-11-3-2 are harvested in bulk. The lines would be designated AX1214-2-2-1 and AX1214-11-3-2.

GENETIC CONSIDERATIONS

The genetic principles of importance in pedigree selection relate to genetic variability expressed among and within lines during inbreeding (Table 25-1). Variability associated with dominance and dominant types of epistasis cannot be utilized in inbred lines. Variability due to additive types of epistasis generally is much smaller than the additive portion of total genetic variability.

Dominance can complicate the selection of homozygous and homogeneous lines by making homozygous dominant and heterozygous individuals indistinguishable. Hybrid vigor expressed by heterozygous individuals also can cause difficulty in selection. When selection of the most vigorous and productive plants in a line favors heterozygous individuals, the progress toward homozygosity is slowed.

MERITS OF THE PEDIGREE METHOD

Advantages

1. If selection is effective, inferior genotypes may be discarded before inbred lines are evaluated in expensive replicated tests.
2. Selection in each generation involves a different environment, which provides a good opportunity for genetic variability of important characters to be expressed and effective selection to be practiced.
3. The genetic relationship of lines is known and can be used to maximize genetic variability among lines retained during selection.

Disadvantages

1. The pedigree method cannot be used in environments where genetic variability for characters is not expressed. This may prevent the use of off-season facilities, with an associated increase in the length of time for cultivar development compared with other methods of inbreeding.
2. A considerable amount of record keeping is involved.
3. Experienced persons may be required to make the necessary selections.
4. The method requires more land and labor than other methods of inbreeding.

REFERENCES

Fehr, W. R. 1978. Breeding. pp. 120–155. *In* A. G. Norman (ed.), *Soybean physiology, agronomy, and utilization*. Academic Press, New York.

Newman, L. H. 1912. *Plant breeding in Scandinavia*. Canadian Seed Growers Association, Ottawa.

CHAPTER TWENTY-SIX

Early-Generation Testing

Early-generation testing is used in self- and cross-pollinated species to estimate the genetic potential of an individual, line, or population at an early stage of inbreeding. The objective is to eliminate lines or populations that do not merit consideration for further inbreeding and selection.

In self-pollinated species, early-generation testing was considered a method of identifying bulk hybrid populations that would contain superior inbred lines. Immer (1941) described the concept on the basis of his work with barley crosses. He indicated that replicated tests of segregating populations in the F_2 or F_3 generation would provide the average yield performance of the different crosses. The high-yielding crosses would be expected to have the highest proportion of high-yielding genotypes. The selected populations would be inbred by the bulk method until the desired level of homozygosity was achieved.

A second concept of early-generation testing has been to identify F_2 plants with superior progenies through replicated testing, then select and evaluate homozygous individuals from within superior F_2-derived lines. This method of early-generation testing was first used by H. Nilsson-Ehle in Sweden.

The concept of early-generation testing for combining ability was proposed by Jenkins (1935) on the basis of his studies on the effects of inbreeding in maize. Until these studies, the common practice for inbred line development in maize was to self and select for multiple generations until uniform inbred lines were obtained. Then the inbred lines were crossed to a common tester to determine their combining ability. Jenkins' study was based on 14 inbred lines from each of two maize populations. Seed of each line in each selfing generation had been saved, which permitted Jenkins to compare the change in combining ability of an inbred as an S_0-derived line, S_1-derived line, and so forth. Each of the inbred lines at each generation of inbreeding was crossed to the common tester and the performance of the testcross (topcross) progeny was evaluated. Jenkins

observed that the relative combining ability of the 28 inbred lines was established already in the $S_{0:1}$ lines. This meant that the inbreds with poor combining ability could have been discarded in early generations.

IMPLEMENTATION

Development of Self-Pollinated Cultivars

Evaluation of Bulk Hybrid Populations. The procedure for early-generation testing of bulk hybrid populations in self-pollinated species begins with the formation of segregating populations and is completed when homogeneous lines are available for testing (see the description below). The populations generally are evaluated as soon as enough seeds are available for testing, which may occur as early as the F_2. The number of locations, years, and replications of testing depends on the characters of interest. Characters with a low heritability and a large genotype × environment interaction would require more extensive evaluation than characters with a higher heritability and smaller genotype × environment interaction. After population selection, inbreeding is continued until the desired level of homozygosity has been achieved, then individual plants are selected. The lines derived from the plants are evaluated for their potential as new cultivars in the same manner as lines derived by other breeding methods.

Season 1: F_2 seeds are obtained from a number of segregating populations.
Season 2: If adequate F_2 seeds are available, the hybrid populations can be evaluated for yield in replicated tests. Populations with poor performance are discarded. If adequate F_2 seeds are not available, the populations are increased by growing F_2 plants and harvesting the F_3 seeds of each population in bulk.
Season 3: The populations are evaluated by planting the F_3 seed in replicated tests. Populations with poor performance are discarded.
Season 4: F_4 seeds of selected populations are planted, and individual plants are harvested.
Season 5: The $F_{4:5}$ lines are evaluated for the characters of interest.

The seed obtained during evaluation of a population may or may not be used for advancing the population to homozygosity. The breeder would use seed from the testing program if it was not excessively contaminated by seed mixtures or outcrossing. Use of seed from test plots would mean that the bulk method of inbreeding was being utilized.

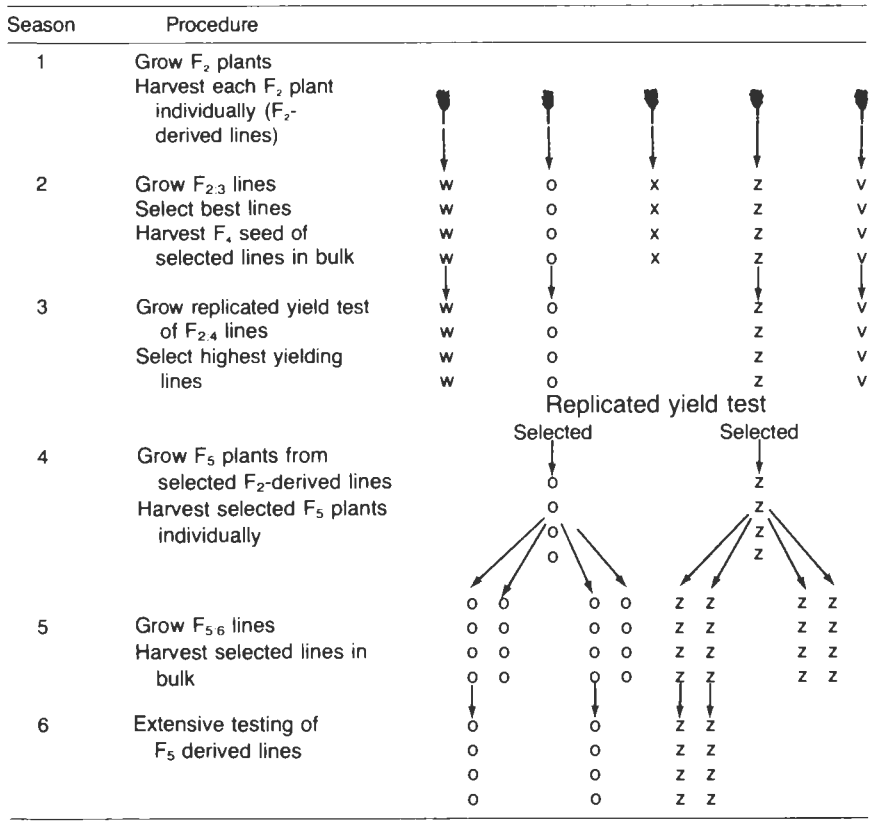
A breeder would not use the seed from the testing program to advance the population if seed mixtures or outcrossing was a problem or if a method of inbreeding other than the bulk method was preferred. Any method of inbreeding could be used in an independent program, including single-seed descent or pedigree.

Evaluation of Lines Derived in Early Generations. The lines evaluated in a program of early-generation testing generally are derived in the F_2 generation (Fig. 26-1). This form of early-generation testing has been referred to as the F_2-derived line method or F_2 family method. In some breeding programs, self-pollinated cultivars are developed and released as F_2- or F_3-derived lines. The evaluation of lines derived in early generations that are released as cultivars per se is not considered early-generation testing. Early-generation testing occurs when heterozygous, heterogeneous lines are evaluated and homozygous plants selected from the superior heterogeneous ones are the source of lines for consideration as potential new cultivars (Fig. 26-1):

Season 1: The F_2 generation is grown, and plants with desirable characteristics are harvested individually from each population.

Season 2: $F_{2:3}$ lines are grown, and lines with desirable characteristics are harvested in bulk.

Figure 26-1 Diagrammatic representation of early-generation testing of F_2-derived lines. (Courtesy of Fehr, 1978.)



Season 3: $F_{2:4}$ lines are evaluated in replicated yield tests, and lines with inferior performance are discarded.

Season 4: F_5 seeds of selected F_2-derived lines are planted, and individual plants with desirable characteristics are harvested individually from each line.

Season 5 + : The F_5-derived lines are evaluated for the characters of interest.

The breeder has a number of options in a program of early-generation testing.

1. The generation in which lines are derived for the early test can vary, usually being the F_2 or F_3.
2. The generation in which the lines are tested generally depends on when enough seed can be obtained and the possibility of discarding lines visually before a more extensive and expensive evaluation is conducted in replicated plots. If enough seed for an early-generation test is obtained from a single plant, the evaluation can begin the next generation. If seed from a single plant is not adequate, a season must be devoted to seed increase. Some breeders prefer to grow the lines for a generation of visual selection before they conduct replicated tests, even if a seed increase is not needed.
3. The number of replications, locations, and years of testing depends on the heritability of the trait, the influence of genotype × environment interaction, and the amount of resources available. With fixed resources, a breeder must find a balance between the testing of heterogeneous lines and the testing of pure lines derived from the superior heterogeneous ones.
4. The breeder must decide if selfed seed obtained from the plots used for replicated testing of a line will be adequately pure, or if an independent program of inbreeding must be conducted for each line.
5. The selected lines can be advanced to the desired level of homozygosity by the bulk, single-seed descent, or pedigree method.
6. The number of plants chosen from each selected heterogeneous line may vary from one to many. The greater the number, the better the opportunity to sample the genetic variability within a line. With fixed resources, however, it is necessary to find a balance between the number of heterogeneous lines from which selections will be made and the number of plants selected from each. The plants selected from within heterogeneous lines are evaluated in the same way as those obtained by any other method.

Development of Inbred Lines for Use in Synthetics or Hybrid Cultivars

Evaluation of Lines per se. Early-generation testing in the development of inbred lines for use in synthetics or hybrid cultivars can include evaluation of F_2- or F_3-derived lines per se. The procedure is the same in principle as that just described for development of self-pollinated cultivars (Fig. 26-1).

In cross-pollinated species, the requirement for artificial self-pollination necessitates that separate programs be carried out for replicated testing and for inbreeding. Open-pollinated seed from a replicated test would be of no value for inbreeding the lines.

Two factors are involved in the choice of a method for inbreeding lines of a cross-pollinated species in a program of early-generation testing.

1. Sampling the genetic variability within a heterogeneous line requires that as many plants as possible be artificially self-pollinated within each line.
2. The time and expense of artificially self-pollinating a large number of plants for each of a large number of lines may exceed available resources. The breeder must find a workable balance between the number of lines to be maintained and evaluated in the inbreeding program and the number of plants to be self-pollinated in each line.

Another consideration for the inbreeding program is the method of handling the seed from self-pollinated plants. If only one plant per line is self-pollinated, there is no decision to make; seed from the plant is harvested and used to plant a progeny row the next season. If a line has two or more selfed plants, the choice for handling the seed is the bulk, single-seed descent, or pedigree method. Each method would be applied to a line in the same manner as that used for a population. For the bulk method, seed from all selfed plants would be bulked and a sample of the bulk would be used to plant the line for the next generation of inbreeding. For single-seed descent, one or a few selfed seeds from each plant would be bulked and used to plant the next generation of the line. For the pedigree method, each selfed plant would be harvested separately and a progeny row grown the next generation. The advantages and disadvantages of each of the methods for inbreeding populations also apply to their use for inbreeding heterogeneous lines.

Evaluation for General Combining Ability. Early-generation tests for general combining ability involve the crossing of individual plants or lines with a tester and evaluation of the performance of the hybrid progeny (see the description below). Separate programs for evaluation and inbreeding must be carried out for each genotype being considered.

Season 1: S_0 plants from a population are self-pollinated to obtain S_1 seed, and are crossed to a tester to obtain testcross seed.

Season 2: Separate programs for evaluation and inbreeding are carried out in season 2:

a. The testcross seed from each S_0 plant is used to plant a replicated test for evaluation of the characters of interest. The S_0 plants with superior testcross performance (superior combining ability) are identified.
b. S_1 progeny from each S_0 plant are grown, and S_1 plants are self-pollinated to obtain S_2 seed.

Season 3: S_2 progeny of S_0 plants with superior combining ability are grown, and the inbreeding process is continued.

A major decision to be made in establishing a program of early-generation testing for combining ability is the number of generations of selfing and selection that will be carried out before genotypes are crossed to a tester. The earliest evaluation of combining ability that can be made is to cross S_0 plants to a tester and evaluate their testcross progeny. The progeny with superior combining ability are retained for additional inbreeding and the progeny with inferior testcross performance are discarded.

The evaluation of combining ability can be delayed until $S_{0:1}$ or $S_{1:2}$ lines with acceptable characteristics have been identified. Acceptable lines may be determined by visual examination in unreplicated plots or by replicated evaluation of lines per se. This delays evaluation for combining ability but eliminates inferior genotypes before expensive testcross trials are conducted. Acceptable selfed plants or lines are crossed to a tester and their testcross progeny are evaluated in subsequent seasons. The following is a description of a program of early-generation testing based on performance of lines per se and for combining ability of $s_{0:1}$ lines. Inbreeding of the lines is carried out by the pedigree method.

Season 1: S_0 plants from a population are self-pollinated and harvested individually. Selection for desired characters is practiced among S_0 plants.

Season 2: $S_{0:1}$ lines are grown in (a) a selfing and selection nursery, (b) a nursery for evaluation of pest resistance, and (c) a nursery to produce testcross seed.

a. Pedigree selection is practiced among and within $S_{0:1}$ lines. Selected plants within agronomically desirable rows are self-pollinated. At harvest, $S_{0:1}$ lines with desirable agronomic characters and pest resistance are chosen. Self-pollinated plants are harvested individually within selected rows.

b. $S_{0:1}$ lines per se are evaluated for pest resistance. Lines with desirable levels of resistance are considered for selection in the selfing nursery. No seed is harvested from the nursery in which pest resistance is evaluated.

c. The $S_{0:1}$ lines and an appropriate tester are grown in a crossing nursery, and testcross seed is produced. Testcross seed is harvested and saved only from those $S_{0:1}$ lines from which selfed seed was harvested the same season.

Season 3: Three separate plantings are made with the seed harvested in season 2. The S_2 progeny from selected $S_{0:1}$ lines are grown in (a) a selfing and selection nursery and (b) a nursery for evaluation of pest resistance. (c) The testcross seed from each $S_{0:1}$ line is used to plant a replicated test.

a. Pedigree selection is practiced among and within $S_{1:2}$ lines. Selected plants within agronomically desirable rows are self-pollinated. At harvest, $S_{1:2}$ lines are chosen with desirable agronomic characters and pest resistance. Self-pollinated S_2 plants are harvested individually within selected rows.

b. $S_{1:2}$ lines per se are evaluated for pest resistance. Lines with desirable levels of resistance are considered for selection in the selfing nursery. No seed is harvested from the nursery in which pest resistance is evaluated.
c. A replicated test for yield and other important characters is conducted and the $S_{0:1}$ lines with the best testcross performance are identified.

With the results from season 3, $S_{0:1}$ lines with superior combining ability and with acceptable agronomic characters and pest resistance are identified. S_3 progeny of the selected lines will be grown in season 4 to continue the inbreeding process.

The number of replications, locations, and years of evaluation for an early-generation test is the option of the breeder. With fixed resources for testing, a balance must be found between tests in early generations and those for more highly inbred lines.

A program for inbreeding is carried out concurrently with evaluation for combining ability. The factors to be considered in the inbreeding program are those discussed in the previous section on the early-generation testing of lines per se.

Relationship of Early-Generation Testing to Recurrent Selection

One of the most important uses of early-generation testing is for recurrent selection. The evaluation of plants or lines with a minimal amount of inbreeding often provides an opportunity for the largest genetic gain per year (Chap. 17). Evaluation of $S_{0:1}$ lines per se is a popular method of recurrent selection in both self- and cross-pollinated species (Chap. 15). The mating of S_0 plants to a tester for evaluation of combining ability is a technique widely used for both intrapopulation and interpopulation improvement of cross-pollinated species. The superior individuals or lines identified as part of a recurrent selection program can be inbred for potential use as a self-pollinated cultivar or as a parent of a synthetic or hybrid cultivar.

GENETIC CONSIDERATIONS

Genetic variability among and within inbred lines is an important consideration in early-generation testing. The additive genetic variability expected for $F_{2:3}$ or $S_{0:1}$ lines is σ_A^2 among and 1/2 σ_A^2 within lines. If selfed progeny of F_2 or S_0-derived lines are maintained in bulk and no natural or artificial selection is practiced, the additive genetic variability among lines will remain σ_A^2, while the average variability within lines increases to 3/4 σ_A^2 for $F_{2:4}$ or $S_{0:2}$ lines and to 7/8 σ_A^2 for $F_{2:5}$ or $S_{0:3}$ lines. On a theoretical basis, there would be considerable genetic variability available for selection among homozygous plants in F_2- or

S_0-derived lines. The number of plants evaluated from each line will determine the extent to which the variability within lines is sampled.

MERITS OF EARLY-GENERATION TESTING

Advantages

1. Inferior individuals, lines, or populations are identified and discarded early in the inbreeding process.
2. More than one cultivar may be derived from a population or heterogeneous line identified as being superior by early-generation testing.

Disadvantages

1. With fixed resources, use of testing facilities for evaluation of individuals, lines, or populations in early generations reduces the number of more highly inbred lines that can be evaluated.
2. The testing program may delay the length of time required for cultivar development.

REFERENCES

Fehr, W. R. 1978. Breeding. pp. 120–155. *In* A. G. Norman (ed.), *Soybean physiology, agronomy, and utilization*. Academic Press, New York.

Immer, F. R. 1941. Relation between yielding ability and homozygosis in barley crosses. *J. Am. Soc. Agron.* 33:200–206.

Jenkins, M. T. 1935. The effect of inbreeding and of selection within inbred lines of maize upon the hybrids made after successive generations of selfing. *Ia. State Coll. J. Sci.* 6:429–450.

CHAPTER TWENTY-SEVEN

Homozygous Lines from Doubled Haploids

The development of uniform inbred lines by conventional methods involves selfing and selection within a population for several generations. The production of homozygous diploid lines by doubling of the chromosome complement of haploid individuals is an alternative method of inbred line development.

The principle of doubled haploids is the same regardless of the method used. Haploid gametes or plants are produced from a heterozygous source. The chromosome complement of the haploids is doubled; therefore, each diploid individual is completely homozygous at all loci. For doubled haploids to be used advantageously in a breeding program, efficient procedures must exist for producing and identifying a large number of haploids and for doubling the chromosome number of the haploids. It also should be possible to produce haploids from any genotype desired. Alternative procedures for producing doubled haploids will be reviewed.

NATURALLY OCCURRING HAPLOIDS

The development of individuals from gametes in the absence of fertilization is referred to as parthenogenesis. Naturally occurring haploids can be parthenogenically derived from both maternal and paternal gametes.

The spontaneous doubling of chromosome number can occur in the tissue of naturally occurring haploids. In maize, approximately 10 percent of haploids produce selfed seed, primarily as a result of spontaneous doubling (Chase, 1949a). The rate of chromosome doubling can be enhanced by the exposure of haploids to certain chemicals or environmental conditions.

Maternally Derived Haploids

The use of naturally occurring haploid individuals was proposed by Chase (1952) as a method of obtaining homozygous diploid lines of maize. Haploids occur in maize at a rate of approximately 1 per 1000 diploids. About 99 percent of these haploids result from the parthenogenic development of haploid cells of the female gametophyte. Various methods of differentiating haploid individuals have been developed on the basis of genetic, cytological, and morphological differences between diploids and haploids.

To obtain a genetically diverse and agronomically desirable diploid line, the production of haploids should involve as the female parent an agronomically desirable F_1 hybrid or a segregating population. The female parent should be the same type of source population that would be used for development of inbred lines by conventional selfing methods. F_1 hybrid plants can be used because their female gametes will be segregating.

To utilize maternally derived haploids, haploid individuals must be distinguishable from diploids produced by outcrossing or self-pollination. Diploid progeny resulting from cross-pollination with the selected male can be distinguished from potential haploids if the male parent used in crossing is homozygous dominant for a marker allele and the female parent is homozygous recessive (Table 27-1). In this case, progeny exhibiting the dominant phenotype for the marker allele would be diploid progeny obtained by fertilization of the female

Table 27-1 Use of Genetic Markers to Identify Maternally Derived Haploids Derived from an F_1 Hybrid

	Genotype		
Individual	Seedling	Endosperm	Phenotype
Parents			
Female-F_1 hybrid	*aa*	*bbb*	Recessive phenotype in seed and seedling
Male	*AA*	*BBB*	Dominant phenotype in seed and seedling
Progeny			
Haploid	*a*	*Bbb*	Dominant phenotype in endosperm and recessive phenotype in seedling
Diploid from self-pollination or outcrossing to recessive male	*aa*	*bbb*	Recessive genotype in seedling and endosperm
Diploid from crossing to selected male	*Aa*	*Bbb*	Dominant phenotype in seedling and endosperm

and male gametes. Progeny with the recessive marker phenotype could be haploids or diploids produced by self-pollination or accidental outcrossing to a male recessive for the marker allele.

The earlier in development a marker is expressed, the less time and resources are required for haploid identification. Chase (1952) suggested the use of purple plant color to distinguish diploid and haploid progeny in maize. The female parent would have the recessive alleles for normal green color (*abp1r*) and the male parent the dominant alleles for purple color (*ABP1R*). Seeds from the cross would be germinated and the seedlings examined for plant color. Normal green seedlings would be potential haploid seedlings. Green seedlings could also be the result of self-pollination or accidental cross-pollination.

Distinguishing haploids from diploids produced by self-pollination or outcrossing to a male recessive for the marker allele requires the use of a marker allele expressed in the endosperm (Table 27-1). The endosperm of both haploid and diploid progeny will contain two genomes from the female parent and one genome from the male parent. If the female parent is homozygous recessive for an endosperm characteristic and the male parent is homozygous dominant, haploid progeny will exhibit the dominant marker phenotype and diploid progeny produced by self-pollination or accidental outcrossing to a recessive male will exhibit the recessive marker phenotype. A commonly used endosperm characteristic in maize is purple aleurone color. A desirable female parent would have recessive alleles for colorless aleurone and the male parent would have dominant alleles for purple aleurone color.

Season 1: A heterozygous female parent is mated to a male parent with appropriate seedling and endosperm markers. The seeds are harvested, and those that have endosperm with the dominant marker phenotype of the male parent are saved.

Season 2: The seeds are germinated, and seedlings with the dominant marker of the male are discarded. Plants with the recessive marker of the female are evaluated for chromosome number by microscopic examination of root tip cells. Plants with the haploid chromosome number are maintained. This often requires transplanting them to the field or to pots of appropriate size or a bench in the greenhouse.

Each haploid plant is self-pollinated. Doubled haploids (diploids) are produced if, through mitotic misdivision, diploid cells of the male and female organs are produced and fertilization occurs. Doubling also may be enhanced by use of chemicals, such as colchicine. Seed is harvested from each plant separately.

Season 3: The seeds obtained in season 2 are planted and the plants are self-pollinated. The plants also may be crossed to a tester to begin the evaluation for combining ability. All subsequent evaluations of the homozygous diploid lines are the same as for lines developed by conventional selfing.

A good male parent for the production of maternal haploids would be one that stimulated above-average production of haploids. Chase (1949b) found that the frequency of haploids varied among male parents.

Paternally Derived Haploids

Haploid seed with the nucleus of the male gamete can develop when the sperm nucleus fails to unite with the egg nucleus. The cytoplasm of the haploid cells is that of the female gamete. The development of paternal haploids is referred to as androgenesis.

Chase (1951) suggested that the production of paternal haploids could be used to transfer cytoplasmic male sterility of maize into male-fertile inbred lines. This would simplify the development of A lines for use as a female parent in hybrid seed production (Chap. 35).

The first report of successful use of androgenesis for converting male-fertile lines to cytoplasmic male sterility was in maize by Goodsell (1961). The frequency of paternal haploids is low; therefore, he used a dominant genetic marker in the cytoplasmic male-sterile female parent to rapidly identify seeds that were paternal haploids (Table 27-2). The cross of a male-sterile, purple root female (*ABP1R*) with a normal white root male (*Abp1r*) would produce F_1 seedlings with purple roots, with the exception of those that did not receive the dominant alleles of the female. The cytoplasm of the haploid would have the male-sterility factors. From such crosses, Goodsell isolated two white-rooted paternal haploid seedlings out of 150,000 progeny screened during one year. Upon pollination with pollen from the male parent, one haploid plant developed seed on diploidized sectors of the ear. The single viable plant derived from this seed was male-sterile. During the following year, Goodsell used this procedure to isolate three additional paternal haploids from approximately 250,000 progeny screened.

Table 27-2 Use of Paternally Derived Haploids in Transferring Cytoplasmic Male Sterility to an Inbred Parent*

Individual	Genetic Marker		Cytoplasm	Origin of Nuclear Genes
	Genotype	Phenotype		
Parents				
Female	*AA*	Dominant	Male sterile	
Male	*aa*	Recessive	Male fertile	
Progeny				
Diploid from fertilization	*Aa*	Dominant	Male sterile	Female and male parent
Paternal haploid	*a*	Recessive	Male sterile	Male parent

*The paternal haploid will have cytoplasmic male sterility from the female parent and the nuclear genotype of the male parent.

One problem with relying on naturally occurring haploids for the development of homozygous diploids is that such haploids occur in a very low frequency. A mutant gene, called the indeterminate gametophyte (*ig*), was observed in maize by Kermicle (1969). It stimulated the production of paternal haploids (androgenesis). The indeterminate gametophyte system was a useful way of enhancing the production of homozygous diploids in a breeding program.

The indeterminate gametophyte gene (*ig*) was a spontaneous mutation in the maize inbred line Wisconsin-23. Kermicle (1969) indicated that when *igig* females were mated with *Ig* males, the frequency of paternal haploids was 23.5×10^{-3}, compared with zero when *IgIg* females were used. The rapid detection of the paternal haploids was accomplished by use of a dominant seed marker in the *igig* female and recessive alleles in the male. Any F_1 seeds lacking the dominant character were found to be haploid (Table 27-2).

Season 1: A female parent with a dominant genetic marker for a seed or seedling character, *igig* alleles, and cytoplasmic male sterility is crossed to a desirable inbred line that has recessive alleles for the genetic marker, has Ig__ alleles, and is male-fertile. F_1 seeds or seedlings with the recessive marker are saved and those that have the dominant marker are discarded.

Season 2: The F_1 individuals with the recessive marker are evaluated for chromosome number, and haploid plants are pollinated with the same inbred parent used as the male in season 1. Seeds will be obtained if a diploid sector develops on the female organ so that normal egg cells are available for pollination.

Season 3: Seeds obtained in season 2 are germinated and cells of their root tips are evaluated for chromosome number. Diploid plants are homozygous for genes of the inbred line used as the male parent and are cytoplasmic male-sterile.

Polyembryony

Naturally occurring parthenogenesis can take place in combination with fertilization to form polyembryonic seeds containing a haploid embryo. Haploid plants derived in this manner can be used to produce homozygous lines. Polyembryonic seeds generally occur at a low frequency; often this is on the order of one in several thousand seeds. Genotypes have been identified in several species, however, that produce a high frequency of polyembryonic seeds. Thompson (1977) obtained 23 twin (diembryonic) seeds out of 215 F_1 progeny of a cross in flax. Most of those twin seeds contained one hybrid embryo and one haploid embryo of maternal origin. Haploid and diploid individuals could be distinguished three days after germination on the basis of seedling size. Thompson outlined an inbred line development program that utilized twin-derived haploids and required half the number of generations for cultivar development as a conventional in-

Table 27-3 Timetable for Proposed Breeding Program that Utilizes Polyembryony (Twinning) of Flax for Inbred Line Production

Time Requirement	Generation*	Technique
4 months	Parent A (twinning line) × Parent B	Cross
4 months	F_1	Self F_1
4 months	F_2	Isolate haploids, rust test, double, collect seed, determine oil content
4 months	G_1	Seed increase in spaced field, winter nursery, determine oil content
4 months	G_2	Summer wilt test, replicated yield tests, etc.
1 year	G_3	Tristate testing
2 to 3 years	G_4, G_5, or G_6	Regional testing
5 to 6 years total	G_6 or G_7	Release cultivar

*The letter "G" represents the generation of plants after the origin of a doubled haploid.
Source: Thompson, 1977.

breeding program (Table 27-3). Genotypes that have a tendency for polyembryony have been identified in Pima cotton (Silow and Stephens, 1944) and pepper (Morgan and Rappleye, 1950).

HAPLOIDS FROM INTERSPECIFIC AND INTERGENERIC CROSSES

Haploids have been found in the progeny of certain interspecific and intergeneric crosses. Haploid production is caused by the interaction of nuclear genes of the species or by nuclear-cytoplasmic interactions.

Chromosome Elimination

The production of a high frequency of haploid individuals in barley has been achieved through crosses between *Hordeum vulgare*, the cultivated species, and *H. bulbosum*, a wild perennial relative (Kasha and Kao, 1970). The homozygous diploids, obtained from doubling the chromosome complement of the haploid, are a source of improved pure-line cultivars. Haploid production from this cross was the result of the loss of *H. bulbosum* chromosomes from hybrid embryos, a phenomenon referred to as chromosome elimination.

The use of interspecific hybridization of cultivated barley and *H. bulbosum* for doubled haploid production is commonly referred to as the Bulbosum method. 'Mingo,' a feed barley cultivar grown in Ontario, Canada, was developed by the Bulbosum method (Foster, 1987).

There are three general steps in the development of diploid pure lines of barley from haploids obtained from the interspecific cross: (a) fertilization and embryo development on the female plant, (b) culturing the haploid embryos and producing haploid plants, and (c) doubling the chromosome number to obtain diploid plants. Chromosome elimination will occur regardless of which species is used as female. However, because the cytoplasm of *H. bulbosum* has deleterious effects on plant growth and vigor, *H. vulgare* is generally used as the female parent.

Kasha (1974) provided a description of the Bulbosum method (Table 27-4). The first step in the procedure involves the production of healthy plants of both parents that are flowering simultaneously. *H. bulbosum* is a perennial with winter growth habit that must be given a low temperature (10°C or less) and short-day treatment (8 to 10 hours of light) for about 8 weeks to induce flowering.

Heterozygous plants of diploid *H. vulgare* are emasculated, and 2 days later pollen from diploid *H. bulbosum* is used to pollinate the flowers. Tillers with the pollinated flowers are removed from the plant and placed in a modified Hoagland's solution to enhance embryo development. One to three days later, 1 drop of 75 ppm (parts per million) gibberellic acid is placed in each floret to improve embryo development, percentage seed set, and the percentage of seeds with embryos. The gibberellic acid is applied for 2 or 3 consecutive days. About 1 week later, the embryos are dissected from the seed and placed on an appropriate culture medium in the dark at 22°C. After 1 to 2 weeks, the differentiated embryos are transferred to an environment of 12 hours of light at 22°C. Haploid seedlings in the two- to three-leaf stage are treated for 5 hours with 0.1 percent colchicine,

Table 27-4 General Procedures for Production of Doubled Haploids of Barley from the Interspecific Cross *Hordeum vulgare* × *H. bulbosum*

Days after Emasculation	Procedure	
0	*Hordeum vulgare* 2n = 2× = 14 emasculation	*Hordeum bulbosum* 2n = 2x = 14
	↓	↓
2	Pollination	Collect fresh pollen
	↓	
3–5	GA_3 treatments, 1 drop/floret of 75 ppm GA_3 for 2 or 3 days	
	↓	
14–16	Dissect and culture embryos, place vials in dark at 22°C	
	↓	
22–28	Transfer differentiated embryos to 12 h light at 22°C	
	↓	
40–50	Treat seedlings (2–3 leaf stage) 5 h with 0.1% colchicine, rinse, and place in pots	

Source: Kasha, 1974.

rinsed, and placed in pots. Diploid seeds that develop on the plants are completely homozygous. The diploid individuals are evaluated in subsequent generations for their agronomic characteristics, in the same manner as lines developed by conventional selfing procedures.

Walsh and colleagues (1973) have recommended that the seed of doubled haploid plants not be used in the evaluation of quantitative characters, due to the decreased vigor of plants derived from this seed source. The first generation progenies of doubled haploid plants can be used for the evaluation of qualitative traits.

The percentage of haploids produced by the Bulbosum method has been greatly increased since the procedure was first developed. Table 27-5 shows the frequencies of success obtained by the Welsh Plant Breeding Station for the various steps involved in the Bulbosum method.

A number of ways of utilizing the doubled haploids produced by this method have been proposed. The greatest savings in generations over conventional inbreeding practices occurs when haploids are produced from the F_1 generation. However, haploids may also be produced from later generations, thus allowing additional opportunities for recombination and selection during self-pollination. In addition, the Bulbosum method could be used to produce pure lines from populations improved by recurrent selection or to purify advanced breeding lines.

Chromosome elimination has been found to occur after interspecific hybridization between several other *Hordeum* species and within the genus *Nicotiana*. Chromosome elimination leads to haploid production in wheat × *H. bulbosum*

Table 27-5 Average Success Rates Obtained at the Welsh Plant Breeding Station in 1977–78 for Steps Involved in the Bulbosum Method of Producing Doubled Haploids of Barley

Steps	Success Rate	
	1977	1978
Seed set (% of pollinations)		
Compatible cross	>80%	>80%
Partially incompatible cross	27%	30%
Seeds dissected (% of seed set)	61%	65%
Embryos cultured (% of seeds dissected)	35%	44%
Haploid plants produced (% of embryos cultured)	50%	55%
Hybrid plants produced (% of embryos cultured)	9%	7%
Rate of chromosome doubling	>80%	>80%
Approximate number of doubled haploids produced per 100 florets pollinated (compatible crosses)	9	13

Source: Pickering, 1979.

crosses. This latter phenomenon has had limited success in the production of inbred lines of wheat to date (Barclay, 1975).

Nuclear-Cytoplasmic Interactions

The involvement of nuclear-cytoplasmic interactions in haploid formation after hybridization does not seem to be a widespread phenomenon. This phenomenon has been reported to occur in intergeneric crosses involving the genus *Aegilops* as female and the *Triticale*-derived hexaploid wheat 'Salmon' as male (Tsunewaki et al., 1968). An average of 30 percent of the progeny produced from this cross are haploid and 6.5 percent are twins. The haploid seed and haploid components of twin seed originate primarily from the parthenogenic development of egg cells.

ANTHER AND POLLEN CULTURE

Haploid plants can be produced in some crop species from pollen. Both intact anthers and isolated pollen have been used. By doubling the chromosome number of the haploid plant, the breeder can obtain homozygous individuals for evaluation.

The production of haploid plants from pollen has two steps: (a) development of embryoids or callus from pollen cells and (b) differentiation into plants. The first report of embryoids from anther tissue was by Guha and Maheshwari (1964) working with the weed *Datura innoxia* Mill. Later research indicated that plants that developed from anthers of *Datura* were haploid (Guha and Maheshwari, 1966). The first report of haploid plants from anther culture with a cultivated species was by Bourgin and Nitsch (1967) in France, working with tobacco. Independent research in Japan resulted in the production of haploid plants of tobacco (Nakata and Tanaka, 1968; Tanaka and Nakata, 1969) and rice (Niizeki and Oono, 1971). Subsequent research has led to successful anther culture in many species.

Plants sometimes develop from 2n somatic cells of the anther. This complication can be overcome by the culture of isolated pollen. Kameya and Hinata (1970) obtained cell clusters from pollen cultured in drops of liquid medium. Sharp and colleagues (1971) later obtained haploid callus from pollen grains of tomato using a nurse culture technique. Nitsch (1974) reported the development of tobacco plantlets from pollen cultured on a synthetic medium. This procedure has since been extended to several species.

There is considerable variation among plant species in the techniques that provide the greatest success for recovery of haploid plants from anther culture. The principal factors that must be considered in use of anther culture were reviewed by Sunderland (1974).

Source Plants

In a breeding program, genetic variability among haploid plants is obtained by utilizing heterozygous plants as the source for the anthers. The heterozygous source can be an F_1 plant, a segregating population of F_2 plants, a random-mating population, or an open-pollinated cultivar.

Stage of Anther Development

The recovery of haploid plants from anthers is influenced by the stage of anther development at the time of culture. The critical period seems to be between the tetrad stage and immediately after the first pollen mitosis; however, the optimum stage within that period varies among species.

Culture Environment

The culture medium plays a critical role in the success of haploid formation from anthers. The proper medium and light and temperature conditions cause the pollen grains to undergo cell division to form an embryoid or callus. Embryoid formation is preferred because embryoids readily develop into plants that generally are free of chromosome abnormalities. If callus is obtained, it must be induced to regenerate into plants.

A wide variety of culture media have been utilized in different crop species. Part of the challenge with anther culture is finding the medium that will give the desired results.

Verification of Haploidy

It is common to obtain plants from anther culture that are not haploid, particularly when regeneration occurs from callus tissue. Thus, it is important to evaluate the chromosome number of the plants to identify those that are haploid.

Formation of Diploids

The chromosome number of haploid plants may double spontaneously; however, colchicine commonly is used to induce doubling. The homozygous diploid individuals are evaluated for agronomic potential in the same manner as lines developed by conventional selfing. The basic anther culture technique is that described for tobacco by Nitsch and Nitsch (1969), as follows:

Step 1: Select flower buds at the proper stage of development. For *Nicotiana tabacum,* formation of plantlets occurs when pollen grains are fully in-

dividualized, uninucleate, and devoid of starch. Pollen grains within the anthers of *N. tabacum* are at that stage when flowers have the top of their petals reaching the tip of their sepals.

Step 2: Dip the cut end of the pedicel of the flower into molten paraffin, then disinfect the whole bud by dipping it into 70 percent ethanol and immersing for about 3 minutes in a filtered suspension of 7 percent calcium hypochlorite.

Step 3: Remove the stamens aseptically and plant them on an appropriate nutrient medium.

Step 4: Grow the cultures at 28°C (day) and 22°C (night) under fluorescent lights supplemented with incandescent light giving about 5500 lux/m^2 outside the tubes containing the cultures. After 4 to 6 weeks, the plantlets may be transferred to a medium devoid of indole-3-acetic acid and containing only 1 percent sucrose.

Step 5: After the plantlets have developed a sufficient root system, transplant them with the rest of the agar medium into a mixture of peat and vermiculite (1 : 1 by volume) and water them with a nutrient solution such as Hoagland's. For about 1 week after the transfer to pots, the plants should be covered with a polyethylene bag to prevent desiccation.

GENERAL ADVANTAGES AND DISADVANTAGES OF DOUBLED HAPLOIDS

Advantages

1. Homozygosity is achieved in fewer generations than required in conventional inbreeding programs. This can reduce the time involved in developing inbred lines.
2. Selection among the homogeneous progeny of doubled haploids can be more efficient than selection among and within heterogeneous progeny during conventional inbreeding.
3. A homogeneous source of seed is available for the increase and release of a new cultivar.
4. Selection among haploid plants for characters controlled by a dominant allele is not complicated by the problem of distinguishing between homozygous-dominant and heterozygous diploid individuals.

Disadvantages

1. The savings in time achieved during inbreeding by the production of doubled haploids may be partially offset by an increase in the length of time required for the evaluation of homozygous lines. There is no op-

portunity for phenotypic evaluation during production of doubled haploids. In contrast, breeders have the opportunity to observe line performance in the field at each inbreeding generation in a conventional inbreeding program. By the time a line has been inbred, its field performance may be fairly well characterized and may require less further evaluation than that of doubled haploid-derived lines.

2. The production of doubled haploids may require specialized equipment and personnel.
3. The frequency of haploids may be impossible to predict for a population. In contrast, a breeder can control the number of plants that are inbred from a population by conventional methods.
4. Preferential haploid production from certain genotypes may result in a nonrandom array of doubled haploids from a heterozygous source of gametes.
5. The performance of inbred lines derived as doubled haploids may be inferior to that of inbred lines developed by conventional inbreeding methods.

REFERENCES

Barclay, J. R. 1975. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. *Nature* 256:410–411.

Bourgin, J. P., and J. P. Nitsch. 1967. Obtention de *Nicotiana* haploides á partir d'etamines cultivées *in vitro*. *Ann. Physiol. Veg.* 9:377–382. [English summary]

Chase, S. S. 1949a. Spontaneous doubling of the chromosome complement in monoploid sporophytes of maize. *Proc. Ia. Acad. Sci.* 56:113–115.

———. 1949b. Monoploid frequencies in a commercial double cross hybrid maize, and its component single cross hybrids and inbred lines. *Genetics* 34:328–332.

———. 1951. The monoploid method of developing inbred lines. *Proc. Sixth Ann. Hybrid Corn Indus. Res. Conf.* 6:29–34.

———. 1952. Production of homozygous diploids of maize from monoploids. *Agron. J.* 44:263–267.

Foster, A. E. 1987, pp 83–125. Barley. *In* W. R. Fehr (ed.), *Principles of cultivar development*. Vol. 2. *Crop species*. Macmillan, New York.

Goodsell, S. 1961. Male sterility in corn by androgenesis. *Crop Sci.* 1:227–228.

Guha, S., and S. C. Maheshwari. 1964. *In vitro* production of embryos from anthers of *Datura*. *Nature* 204:497.

———. 1966. Cell division and differentiation of embryos in the pollen grains of *Datura in vitro*. Nature 212:97–98.

Kameya, T., and K. Hinata. 1970. Induction of haploid plants from pollen grains of *Brassica*. *Jap. J. Breed.* 20:82–87.

Kasha, K. J. 1974. Haploids from somatic cells. pp. 67–68. *In* K. J. Kasha (ed.), *Haploids in higher plants*. University of Guelph, Ontario.

Kasha, K. J., and K. N. Kao. 1970. High frequency haploid production in barley (*Hordeum vulgare L.*). *Nature* 225:874–876.

Kermicle, J. L. 1969. Androgenesis conditioned by a mutation in maize. *Science* 166:1422–1424.

Morgan, D. T., and R. D. Rappleye. 1950. Twin and triplet pepper seedlings: A study of polyembryony in *Capsicum frutescens*. *J. Hered.* 41:91–95.

Nakata, K., and M. Tanaka. Induction of haploid rice plant from anther culture. *Proc. Jap. Acad.* 44:554–557.

Niizeki, H., and K. Oono. 1971. Rice plants obtained by anther culture. *In Les cultures de tissus de plantes*. Colloques Internationaux du CNRS No. 193. Paris.

Nitsch, C. 1974. La culture de pollen isolé sur milieu synthetique. *C. R. Acad. Sci. Ser. D.* 278:1031–1034.

Nitsch, J. P., and C. Nitsch. 1969. Haploid plants from pollen grains. *Science* 163:85–87.

Pickering, R. A. 1979. Use of the doubled haploid technique in barley breeding at the Welsh Plant Breeding Station. *Report of the Welsh Plant Breeding Station for 1978*, pp. 84–85.

Sharp, W. R., D. K. Dougall, and E. F. Paddock. 1971. The use of nurse culture in the development of haploid clones in tomato. *Planta* 104:357–361.

Silow, R. A., and S. G. Stephens. 1944. Twinning in cotton. *J. Hered.* 35:76–78.

Sunderland, N. 1973. Pollen and anther culture. *In* H. E. Street (ed.), *Plant tissue and cell culture*. Blackwell Scientific Publishers, Oxford.

———. 1974. Anther culture as a means of haploid induction. pp. 91–122. *In* K. J. Kasha (ed.), *Haploids in higher plants*. University of Guelph, Ontario.

Tanaka, M., and K. Nakata. 1969. Tobacco plants obtained by anther culture and experiments to get diploid seeds from haploids. *Jap. J. Genet.* 44:47–54. [English summary]

Thompson, T. E. 1977. Haploid breeding technique for flax. *Crop Sci.* 17:757–760.

Tsunewaki, K., K. Noda, and T. Fujisawa. 1968. Haploid and twin formation in a wheat strain Salmon with alien cytoplasms. *Cytologia* 33:526–538.

Walsh, E. J., E. Reinbergs, and K. J. Kasha. 1973. The importance of seed source in preliminary evaluations of doubled haploids in barley. *Can. J. Plt. Sci.* 53:257–260.