PRINCIPLES OF CULTIVAR DEVELOPMENT

VOLUME 1

Theory and Technique

Walter R. Fehr

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Theory and Technique

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with the assistance of
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Reprinted 1993

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To my wife Elinor, whose numerous contributions to this book and to my life have been of immeasurable value.

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Preface

The development of superior cultivars of plant species is a challenge that tests the ingenuity, patience, and persistence of an individual. Ingenuity is based on an appreciation of the scientific principles of genetics, agronomy, horticulture, statistics, physiology, and many other disciplines that are an essential part of plant breeding. It involves the ability to evaluate an array of alternative methods for cultivar development, assess the resources that are available, and develop a strategy that is efficient and effective. Patience is required to undertake the development of a cultivar, a process that commonly requires 10 years or more. Persistence is essential in dealing with the numerous obstacles that must be confronted, particularly uncontrollable fluctuations in the weather.

As a university professor, it has been my privilege to teach young women and men who have the ingenuity, patience, and persistence required to be a plant breeder. One of my responsibilities has been to help students understand how cultivar development actually is carried out, sometimes referred to as the nuts and bolts of plant breeding. My colleagues generously shared their experiences with me, which made it possible to develop a set of class notes for distribution to the students. Those class notes became the foundation for this book.

The purpose of the book is to provide some assistance in the decision-making process that every plant breeder encounters. There are not any plant breeding programs that are identical in all respects. Each breeder is faced with unique circumstances for which an appropriate strategy of cultivar development must be developed. The plant species, resources available, expectations of the employer, and demands of the marketplace are a few of the factors that contribute to the circumstances that are encountered. To develop an effective strategy of cultivar development, the breeder must be able to understand the alternative methods that could be used and evaluate the genetic improvement that could be realized from each method. This book is intended to describe in detail the alternative breeding methods and to provide guidelines for the evaluation of their advantages and disadvantages under different circumstances.

The selection and application of plant breeding methods for the genetic improvement of a crop species depends on such factors as the types of cultivars that are grown commercially, the type of parental germplasm available, and the objectives of cultivar improvement. To help students and other interested people understand how plant breeders develop an appropriate strategy of genetic improvement, Volume 2 of *Principles of Cultivar Development* was prepared. In that volume, successful plant breeders describe the step-by-step process of cultivar development for the crop series with which they work, discuss alternative procedures that are available for each step of the process, and provide examples of those methods that have been used most successfully.

There is considerable emphasis in current plant research on the role of cellular and molecular biology in genetic improvement of plant species. The results of the research undoubtedly will improve procedures for cultivar development in the future. The emphasis in this book has been placed on techniques that actually have been used to develop cultivars, however, instead of on future possibilities that have yet to be widely adopted by plant breeders. Future opportunities for the improvement of plant breeding methods are addressed by the authors of individual crop species in Volume 2 of *Principles of Cultivar Development*.

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WALTER R. FEHR

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CHAPTER ONE

Role of Plant Breeding in Agriculture

The development of improved cultivars has made a major contribution to the increased productivity and quality of plants used for their food, feed, fiber, or aesthetic value. Selection of the appropriate cultivar is one of the key decisions that an agricultural producer must make. The cultivar will define the limits of performance that can be achieved in any environment.

Plant breeding is the art and science of the genetic improvement of plants. It has been a part of agriculture since humans first selected one type of plant or seed in preference to another, instead of randomly taking what nature provided. Preferential selection to meet particular human needs resulted in a broad range of cultivated types within a species. There was variation for time of harvest, size and color of seeds and plants, and flavor of the product. Each producer that contributed to the selection of a particular plant type was a practitioner of plant breeding.

The relative importance of art and science in plant breeding has changed over time. The ability of a person to visually identify the most desirable plants or seeds was the only tool available in the beginning of plant selection. The appearance, or phenotype, of a plant determined whether it would be chosen. The act of visually selecting desirable plants remains a part of plant breeding. It is now possible, however, to plan an effective program of genetic improvement based on scientific information. An understanding of heredity facilitates the development of useful genetic variability and the selection of superior individuals. The effectiveness of plant breeding will increase as scientific research expands our knowledge of the genetic basis of plant performance.

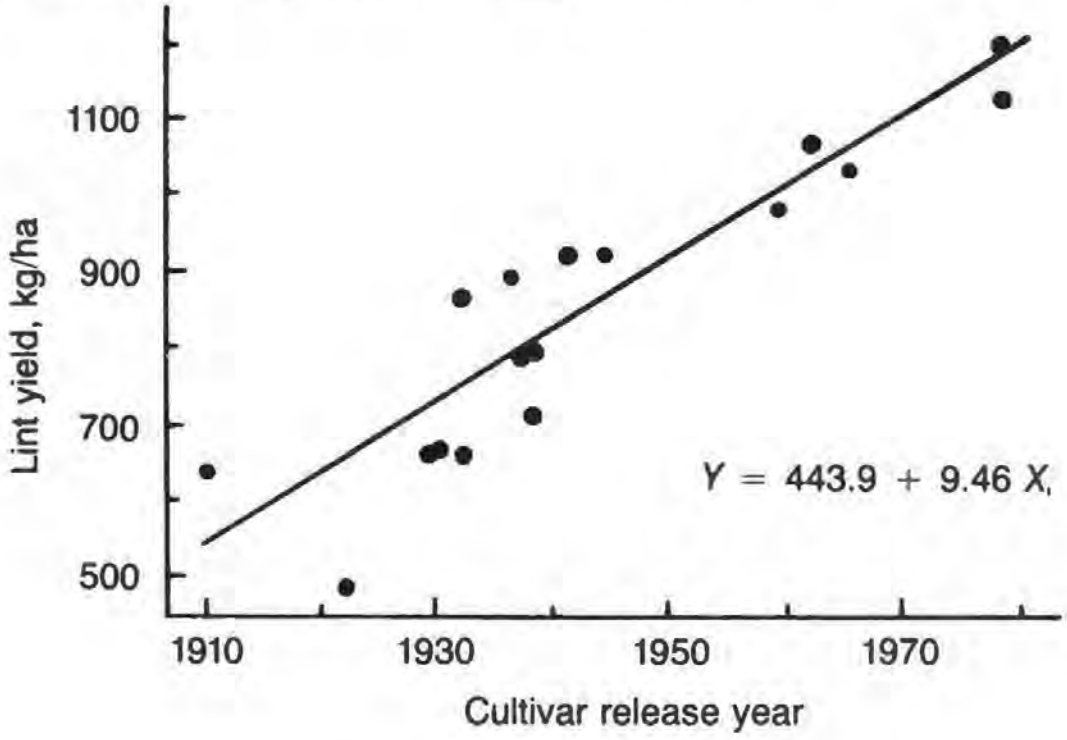
CHARACTERISTICS IMPROVED BY PLANT BREEDING

The overall objective of plant breeding is to improve those characteristics of a species that contribute to its economic value. The part of a plant having economic value may be the leaf, stem, root, flower, fruit, or seed. Selection can be practiced for direct improvement of the plant part or for characters that are related to reliability of production, harvestability, and marketability. The entire list of characteristics considered by plant breeders is too lengthy to reproduce here. The following traits are of primary importance in the improvement of many crop species.

Yield

The amount of production per unit area is a trait of primary importance. Average yields of many crops have increased over time due to higher soil fertility; improved disease, insect, and weed control; more effective plant spacings; and improved cultivars. Numerous examples are available of the role of improved cultivars in increasing the productivity of a crop (Fig. 1–1, 1–2, and 1–3; Table 1–1). In maize, development of double-cross hybrids to replace open-pollinated cultivars during the 1930s was responsible for about a 7 to 12 percent increase

Figure 1–1 Average lint yield (kilograms per hectare) of obsolete and modern cotton cultivars grown at Stoneville, Mississippi, in 1978 and 1979 (Bridge et al., 1982). Y = lint yield and X = years after 1900.



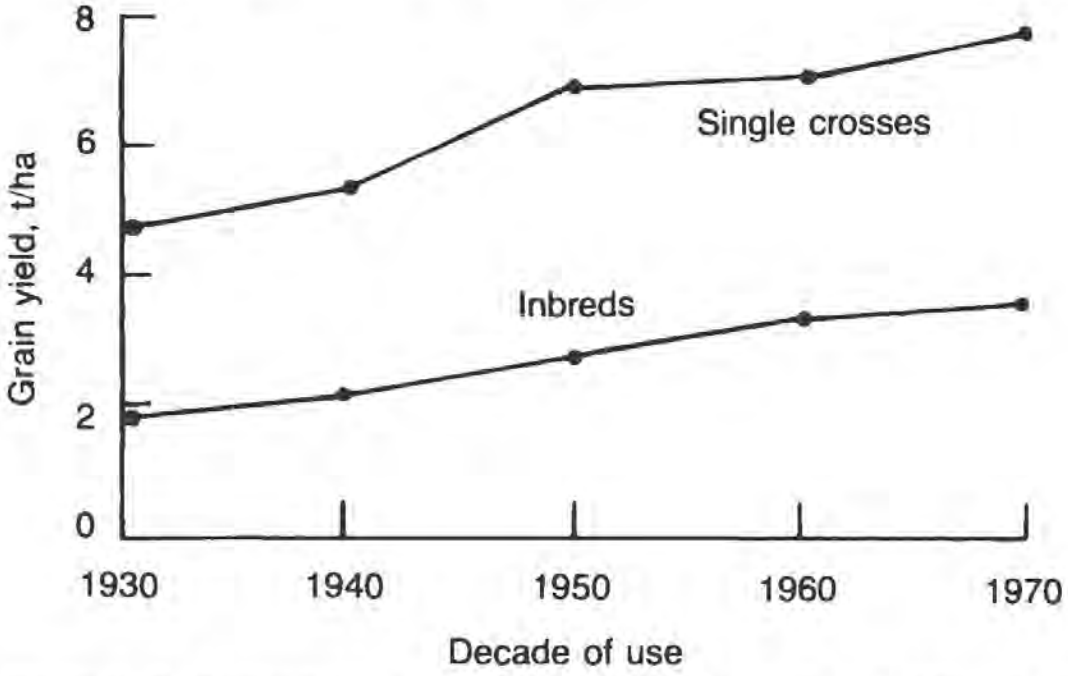


Figure 1–2 Comparison of grain yields (tons per hectare) for single-cross hybrids of maize and their inbred line parents from different decades of use when grown in the same environment. (Courtesy of Duvick, 1984.)

in yield (Frey, 1971; Russell, 1974). Genetic improvement of double-cross hybrids from the 1930s to 1960s resulted in a yield increase of about 28 percent. Adoption of single-cross hybrids in the 1970s resulted in further improvement in yield. The total yield improvement of single-cross hybrids over open-pollinated cultivars has been more than 50 percent.

Dramatic yield improvements in wheat and rice during the 1960s and 1970s played a major role in augmenting world food production, a phenomenon referred to as the Green Revolution. These yield increases were associated with the development of short-statured cultivars that perform exceptionally well in highly productive environments. The international significance of the accomplishment was highlighted when Norman E. Borlaug received the Nobel Peace Prize in 1970 for his contribution to the development and distribution of short-statured wheat cultivars.

Resistance to Pests

Genetic resistance is the most effective means of biological control of diseases, nematodes, and insects. Resistance of cultivars commonly eliminates or minimizes the need for chemical fungicides, nematocides, or insecticides, even though the plants are exposed to pests that have the capability to injure susceptible ones.

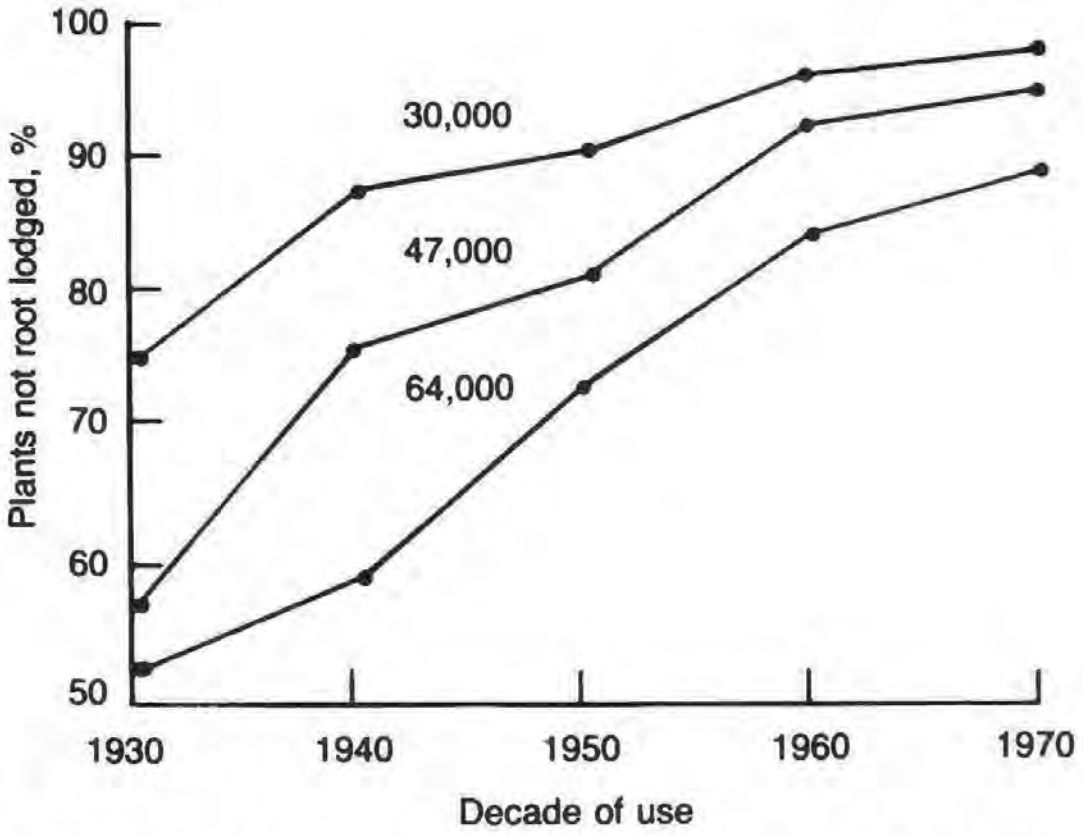


Figure 1–3 Mean percentage of plants without root lodging for single crosses of maize from different decades of use when grown in the same environment at 30,000, 47,000, and 64,000 plants/ha. (Courtesy of Duvick, 1984.)

Selection for resistance to one or more pests is an objective of most cultivar development programs.

Seed Composition

The value of seed can be influenced by its chemical composition. The percentage content and quality of protein in seeds affect their nutritional value as food for humans and livestock. The fatty acid composition of seed oil determines its acceptability as food and as a raw material for industrial uses. Carbohydrate and

Table 1-1 Contribution of Plant Breeding to Yield Improvement of Five Major Crops

Crop	Average Gain per Year	Source
Cotton	7 kg/ha	Meredith and Bridge, 1984
Maize	92 kg/ha	Duvick, 1984
Soybean	18.8 kg/ha	Specht and Williams, 1984
Sorghum	1–2%	Miller and Kebede, 1984
Wheat	0.74%	Schmidt, 1984

sugar content are important considerations in the products obtained from some crops.

Cultivars with increased levels of a chemical component have been developed in several species. The malting barley cultivar 'Morex,' released in 1978, has a high percentage of total malt extract (Rasmusson and Wilcoxson, 1979). The protein in maize kernels is low in the essential amino acids lysine and tryptophan. In Brazil, hybrids with higher lysine content have been grown by farmers, have had good yields, and have improved the nutritional quality of food and feed (Secundino de Sao Jose, 1975). The development of rapeseed cultivars with a low content of erucic acid has improved the value of the oil (Dorrell and Downey, 1964).

Forage Quality

The productivity of cattle, sheep, and other ruminant animals is related to the quality of the forage they consume. The amount of daily gain in the weight of animals who eat the forage is one of the criteria considered in the selection of forage cultivars. Selection for low alkaloid concentration in reed canarygrass has increased the daily weight gain of lambs by up to 95 percent (Marten et al., 1981).

Tolerance to Mineral Stresses

In some areas, crops must be grown on soils with characteristics that are undesirable for plant growth. The undesirable characteristics may be salinity, alkalinity, or a deficiency of iron, zinc, phosphorus, or other nutrients. The planting of cultivars that tolerate a particular stress has increased productivity on marginal soils. Rice cultivars with salt tolerance have produced an average of 2 ton/ha more than nontolerant ones (Ponnamperuma, 1982). Soybean cultivars have been developed that show little yield reduction on calcareous soils on which other cultivars have yield losses of 80 percent or more (Fehr, 1983). Wheat cultivars with tolerance to aluminum toxicity have allowed production of wheat on acid soils without liming (da Silvia, 1976).

Tolerance to Environmental Stresses

Temperature and moisture extremes can cause major reductions in crop productivity and limit a crop's area of utilization. Breeders were able to identify winter-hardy cultivars in wheat and oat, incorporate this trait into high-performing cultivars, and expand the production area of these crops (Marshall, 1982).

Plants within a species may vary in their response to environmental stresses induced by humans. Plant performance can be reduced by excessive concentra-

induced by humans. Plant performance can be reduced by excessive concentrations of air pollutants, particularly around heavily populated and industrialized areas. Breeding for resistance to air pollutants has been effective in reducing the level of injury to plants. 'Santa Ana' is a smog-resistant bermudagrass cultivar developed for California (Youngner, 1966).

Adaptability to Mechanization

The mechanization of some crops has been facilitated by the development of cultivars with modified characteristics of the seed or plant. Sugar beet production in the world involved planting multigerm seed until the mid-1950s. Each seed produced several plants, which had to be thinned to the proper plant density by hand. In 1948, plants with monogerm seeds were identified. Each seed produced only one plant; therefore, plant density could be regulated by seeding density. Nearly all commercial seed used today has the monogerm characteristic (Campbell and Mast, 1971).

The height of sorghum can reach 5 m or more. The tall height is preferred for high forage yields, but combine harvesting of the plants for seed would be extremely difficult. Sorghum breeders utilize four recessive dwarfness genes for developing hybrids of short stature that are well suited to combine harvesting (Quinby and Karper, 1954).

Machine harvesting of wild rice has been hindered by the shattering of seed upon maturity. Both qualitative and quantitative variations in seed retention have been exploited in developing nonshattering wild rice cultivars (Everett and Stucker, 1983).

Standability

The yield of a crop and its ease of harvest can be influenced by its standability, or lodging resistance. Plant susceptibility to lodging is often more severe under conditions of high fertility. Major improvements in crop production have been made by the development of cultivars that remain upright even with high fertility. The development of semidwarf wheat and rice cultivars resulted in dramatic yield increases under high-fertility conditions (Borlaug, 1965; Huang et al., 1972). Their superiority over taller wheat and rice cultivars was due in part to improved lodging resistance.

Photoperiod Response

The geographical area in which a cultivar or species is productive can be limited by the day length it requires for flower initiation. This photoperiod requirement can vary between and within species. Knowledge of photoperiod sensitivities

has enabled breeders to select for increased adaptivity of cultivars to various latitudes. The identification of photoperiod insensitivity in semidwarf rice and wheat has been important in developing widely adapted, high-yielding cultivars (Dalrymple, 1978).

DISCIPLINES THAT CONTRIBUTE TO CULTIVAR DEVELOPMENT

Cultivar development involves the application of knowledge provided by a number of scientific disciplines. The success of plant breeders is associated with their ability to integrate the disciplines into an effective program.

Agronomy, horticulture, and genetics can be considered the central disciplines of plant breeding. The method by which a crop is produced and utilized determines the characters that are important for selection and the conditions under which the characters should be evaluated. Knowledge of the inheritance of a character is basic to the establishment of an effective selection program. Qualitative and quantitative genetics contribute to the understanding of plant behavior in breeding systems. The application of molecular genetics to cultivar development will be an important consideration for the plant breeder in the future.

A working knowledge of plant pathology and entomology is basic to the development of cultivars with genetic resistance to diseases and insects. A plant breeder and plant pathologist or entomologist often work as a team in cultivar development. The plant pathologist or entomologist determines which pests are of economic importance, develops procedures to identify resistant plants, assists in identifying sources of genetic resistance, and collaborates in the screening of segregates from populations in a breeding program.

Statistics is fundamental to the evaluation of yield and other characters that are subject to variable environmental conditions. The identification of cultivars with superior characteristics requires tests that are properly designed and analyzed. The theory of statistical design aids the breeder in identifying the optimum testing system for a given amount of resources.

Biochemistry plays an important role in the development of cultivars with suitable chemical qualities. The market value of some crops is directly related to their chemical composition. Determination of the chemical composition of seed or forage is a routine part of many breeding programs. Biochemistry also is essential to expanding our knowledge of plant physiology and molecular genetics.

Botany has contributed to cultivar development in many ways. Plant physiology, morphology, and anatomy provide insight into plant growth and development. Knowledge of the morphological and physiological traits that influence plant performance is useful for determining characteristics that should be considered during selection and the best developmental stage at which to practice selection. An understanding of the taxonomic relationships among species is especially important when a desired characteristic is not found in the cultivated

species, but is available in a wild species. Botany has provided information on cell structure, differentiation, and function that is essential for understanding plant growth and development. An understanding of the mode of reproduction for a species is needed to select appropriate breeding methods for cultivar development.

SKILLS REQUIRED OF A PLANT BREEDER

The three primary activities carried out by plant breeders are cultivar development, research on germplasm and breeding methods that contribute to effective cultivar development, and the education of future plant breeders. The extent to which a plant breeder is involved in one or more of these activities varies considerably. A person employed by a private company will be responsible primarily for cultivar development. Individuals who work for a public research agency, such as the U.S. Department of Agriculture or a state agricultural experimental station, will devote part or all of their resources to basic research on germplasm and breeding methods. A plant breeder employed by a university may have responsibility for undergraduate and graduate instruction.

To be capable of planning and conducting an effective plant breeding research program, a person needs an appropriate undergraduate and graduate education (Fehr, 1981; Johnson, 1981; Keim, 1981). The education includes a mixture of formal courses, independent research experience in working with an active research program, and informal interaction with students and faculty. Academic courses include plant breeding and those disciplines that contribute to cultivar development. Independent research gives experience in planning, conducting, and interpreting experiments. Participation in an active research program is a means of obtaining practical experience with breeding techniques and methods. Informal interaction with students and faculty provides the opportunity to evaluate new ideas and concepts.

The ability of the plant breeder to manage time, personnel, and money has a direct bearing on the amount he or she is able to accomplish. Management is such an important skill that universities have been encouraged to include courses in it as part of the plant breeding curriculum. Effective management includes the ability to identify persons who have appropriate skills and are able to work with others as a team. The establishment of budgets, acquisition of funds, and allocation of financial resources are part of the managerial role of the plant breeder. Innovations in techniques often are a result of the effort of a plant breeder to obtain maximum benefit from the money available. A skillful manager is able to set research priorities that focus on important objectives and goals (Johnson, 1981). For example, many characteristics can be evaluated in the development of new cultivars. The breeder must be able to determine which characters are of major importance and which do not warrant attention. Plant breeding research often requires close cooperation with scientists in other disciplines.

Effective communication with scientific and nonscientific persons is a valuable skill (Johnson, 1981). A plant breeder must be able to generate financial support for research through written and verbal communication. The reviewers of proposals may be plant breeders, scientists in other disciplines, administrators, or producers of a commodity. The merit of a proposal and how it is presented are often equally important. The results of research must be communicated to producers and scientists. This may be done in the form of an extension publication, a sales brochure, or a referred journal article. An effective plant breeder is able to adapt the presentation for the audience involved.

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CHAPTER TWO

Modes of Reproduction

The breeding procedures used for genetic improvement of a crop species are dependent on the mode by which cultivars are reproduced for commercial plantings. The two general modes of reproduction are sexual and asexual. Sexual reproduction involves the union of male and female gametes derived from the same parent or different parents. Asexual reproduction can occur by the multiplication of plant parts or by seed production that does not involve the union of male and female gametes.

CELL PROCESSES IN REPRODUCTION

Sexual reproduction is based on the process of meiosis, by which the chromosome number of cells in the female and male reproductive organs is reduced by half to form female and male gametes. Meiosis is responsible for the genetic segregation observed in the progeny of heterozygous individuals. Asexual reproduction is based on the multiplication of cells by mitosis. The process of mitosis results in two new cells that are genetically identical to each other and to the cell from which they originated.

Meiosis

The process of meiosis involves megaspore mother cells within the ovule of the pistil and microspore mother cells within the anther (Fig 2-1). Reductional division by meiosis of the 2n megaspore mother cell to form four haploid (n) megaspores is known as megasporogenesis, while division of the 2n microspore mother cell to form four haploid microspores is referred to as microsporogenesis.

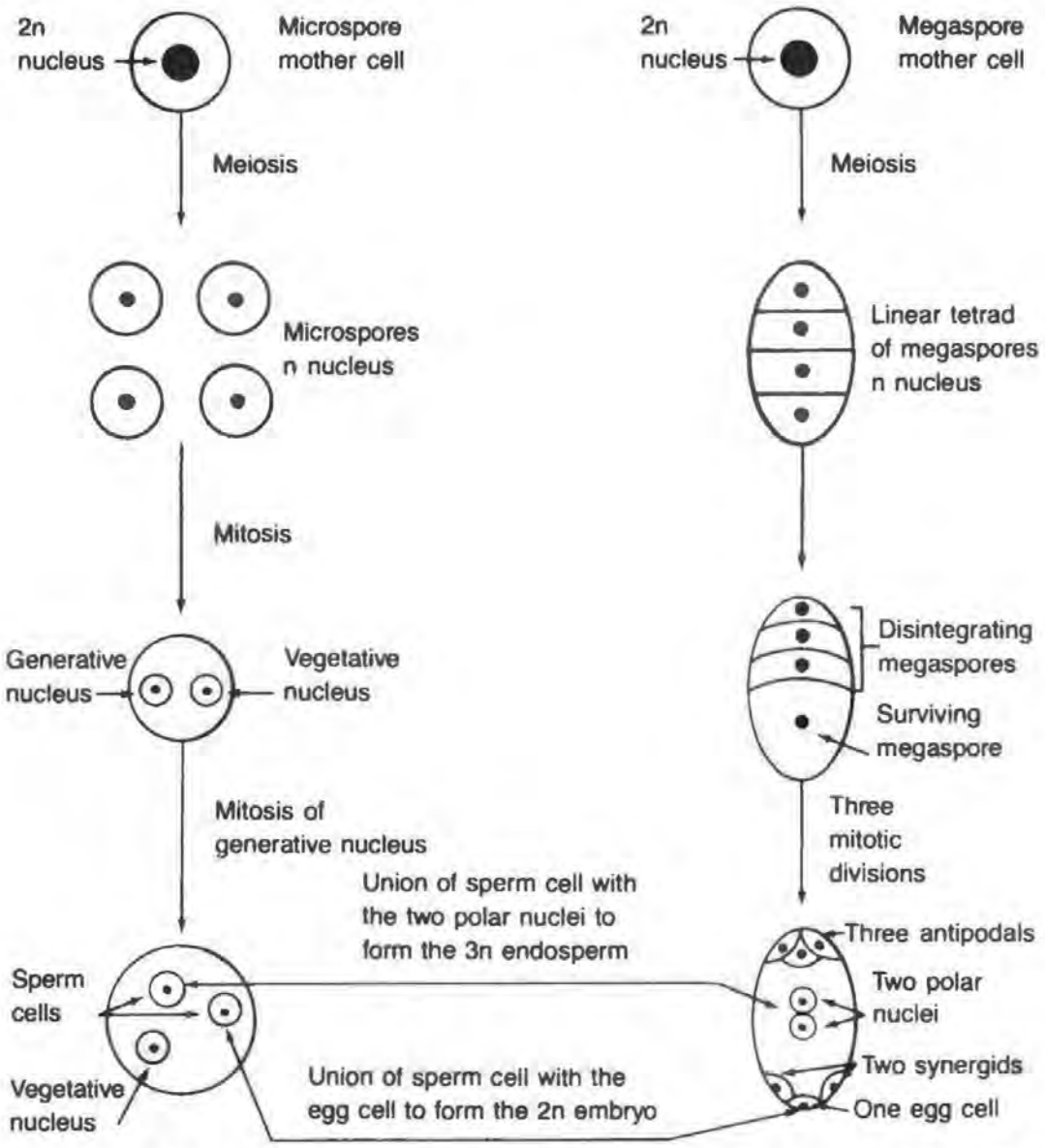


Figure 2-1 General features of the formation of an embryo sac in the ovule and pollen grains in the anther. Fertilization of a haploid (n) egg cell with a sperm results in the formation of the embryo (2n) of the seed. The endosperm tissue (3n) of the seed arises from union of two haploid polar nuclei with the sperm cell.

To facilitate the description of meiosis, the process has been divided into a series of stages. Meiosis I is the first division of the megaspore or microspore mother cell to form two cells, each of which divides during meiosis II to produce a total of four cells. Each of the two general stages is further subdivided into prophase, metaphase, anaphase, and telophase (Fig. 2-2).

Prophase I. The prophase of meiosis I has been divided into five stages (Fig. 2-2).

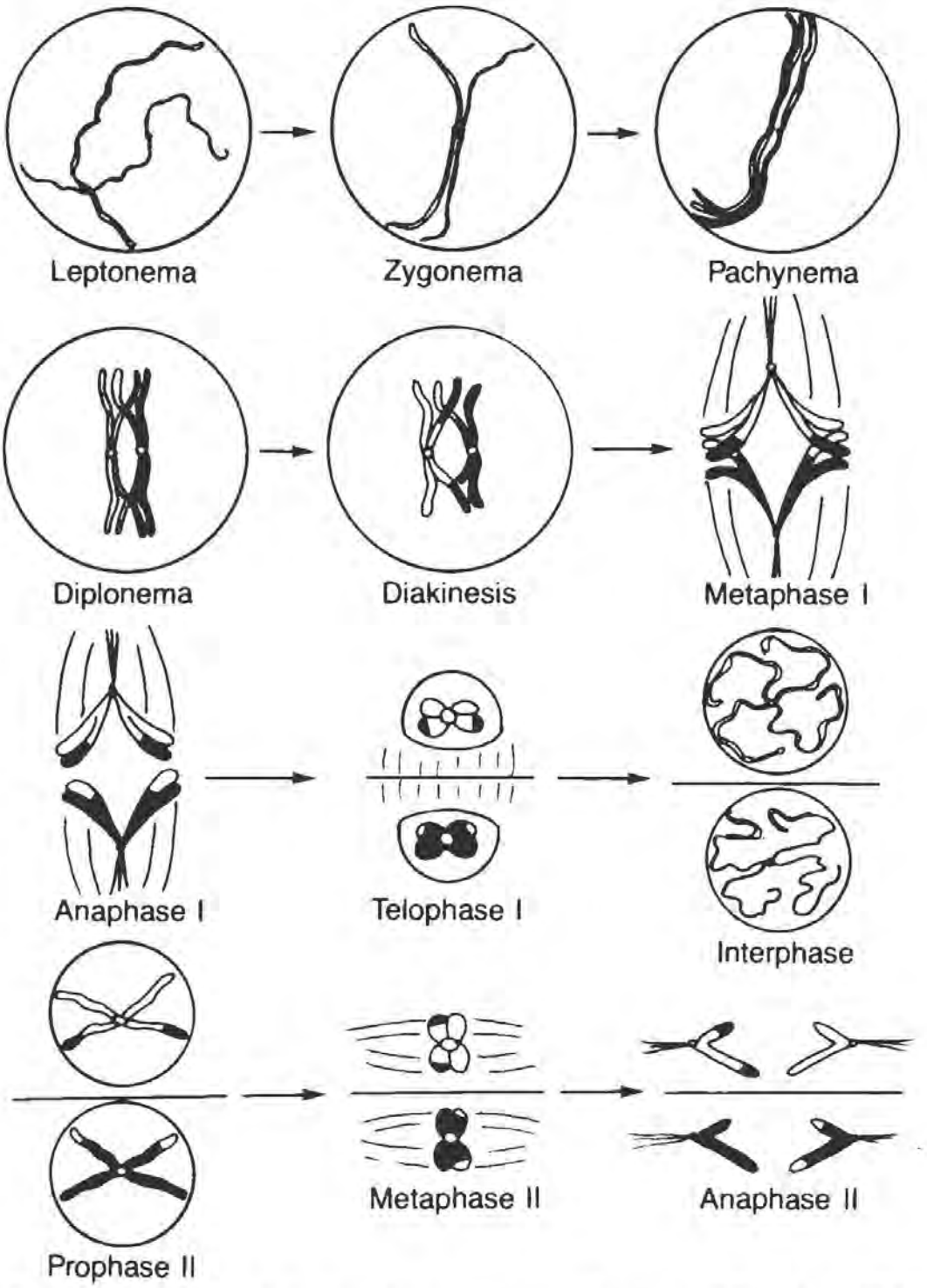


Figure 2-2 Stages of division during meiosis. (Courtesy of Rhoades, 1950.)

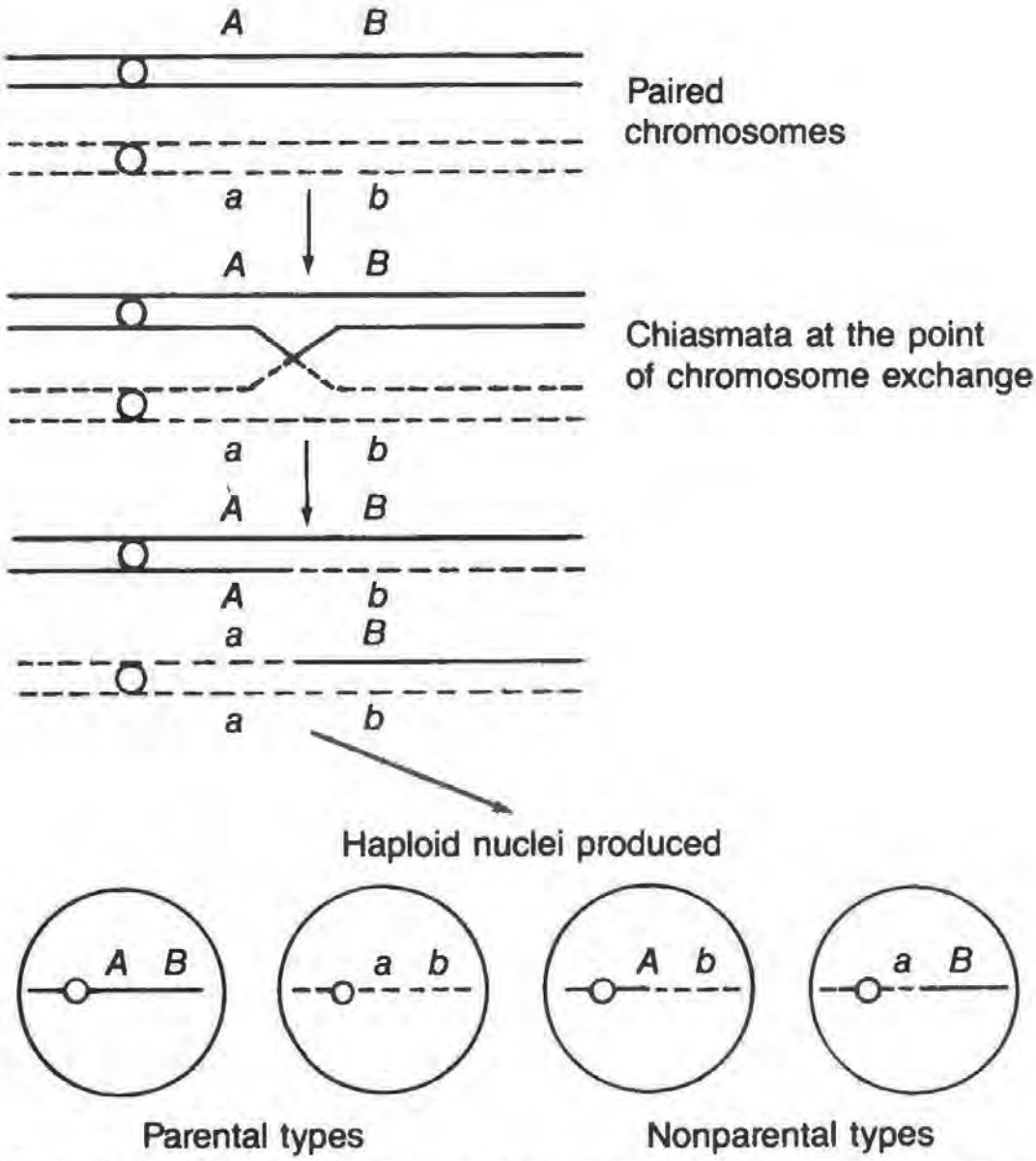


Figure 2-3 Crossing over between paired homologous chromosomes during meiosis resulting in recombination between linked genes. Exchange of chromosome segments can involve any two of the chromatids of the paired chromosomes.

1. *Leptonema:* The chromosomes have a thin, threadlike appearance. The DNA of each of the chromosomes has replicated, and each chromosome consists of two identical members (chromatids).
2. *Zygonema:* The homologous chromosomes of the genome pair (synapse) with each other. A diploid species with $2n = 20$ chromosomes would form 10 pairs of homologous chromosomes during zygonema.
3. *Pachynema:* Crossing over occurs between the chromatids of homologous chromosomes (Fig. 2-3). The genetic recombination that occurs during

crossing over in a heterozygous individual is responsible for part of the segregation observed in its progeny.

4. *Diplonema:* The homologous chromosomes begin to separate, but are held together by chiasmata. Chiasmata are formed as a result of crossing over.
5. *Diakinesis:* The nuclear wall disappears and the chromosomes are coiled to their shortest length.

Metaphase I. The chromosomes of the genome line up within the cell at a position referred to as the equatorial plate (Fig. 2-4). Spindle fibers form, which link each chromosome of a homologous pair to a different pole in the cell. The orientation of the chromosomes relative to the two poles seems to be random in a normal cell. Furthermore, the orientation for each homologous pair in a genome is independent of that of any other pair. The random orientation of the chromosomes of a genome results in different genetic combinations in the gametes of heterozygous individuals. In Fig. 2-4, the orientation of two chromosome pairs at metaphase I results in chromosomes with the *A* and *B* alleles going to one pole and chromosomes with the *a* and *b* alleles going to the other. An equally possible alternative would be chromosomes with the *A* and *b* alleles going to the one pole and chromosomes with the *a* and *B* alleles going to the other. The number of different combinations of chromosomes that can occur due to their orientation at metaphase I is defined by the formula 2^{n-1}, where n is the number of chromosomes in the genome. There are two combinations possible with two chromosome pairs, four combinations with three chromosome pairs, and eight combinations with four chromosome pairs.

Anaphase I. The homologous chromosomes separate and move to opposite poles of the cell. The two chromatids of each chromosome, which are attached at the centromere, remain together during this stage.

Telophase I. Telophase I marks the end of the first phase of meiosis. The haploid chromosome number has been established, because only one member of a homologous pair in a genome is present at each of the two poles of the cell. A nuclear membrane may form and the chromosomes may uncoil during this stage. A cell plate develops to separate the two cells formed by meiosis I.

Interphase. Interphase represents the period between meiosis I and II. Its duration varies considerably among species.

Meiosis II. Each cell formed by meiosis I undergoes division to produce two identical cells during meiosis II, a process that resembles mitosis (Fig. 2-2 and 2-4). The chromosomes coil and become shorter during prophase II. During metaphase II, the chromosomes line up at the equatorial plate and spindle fibers attach to each of the two chromatids. The chromatids of each chromosome separate and move toward opposite poles at anaphase II. At telophase II, the

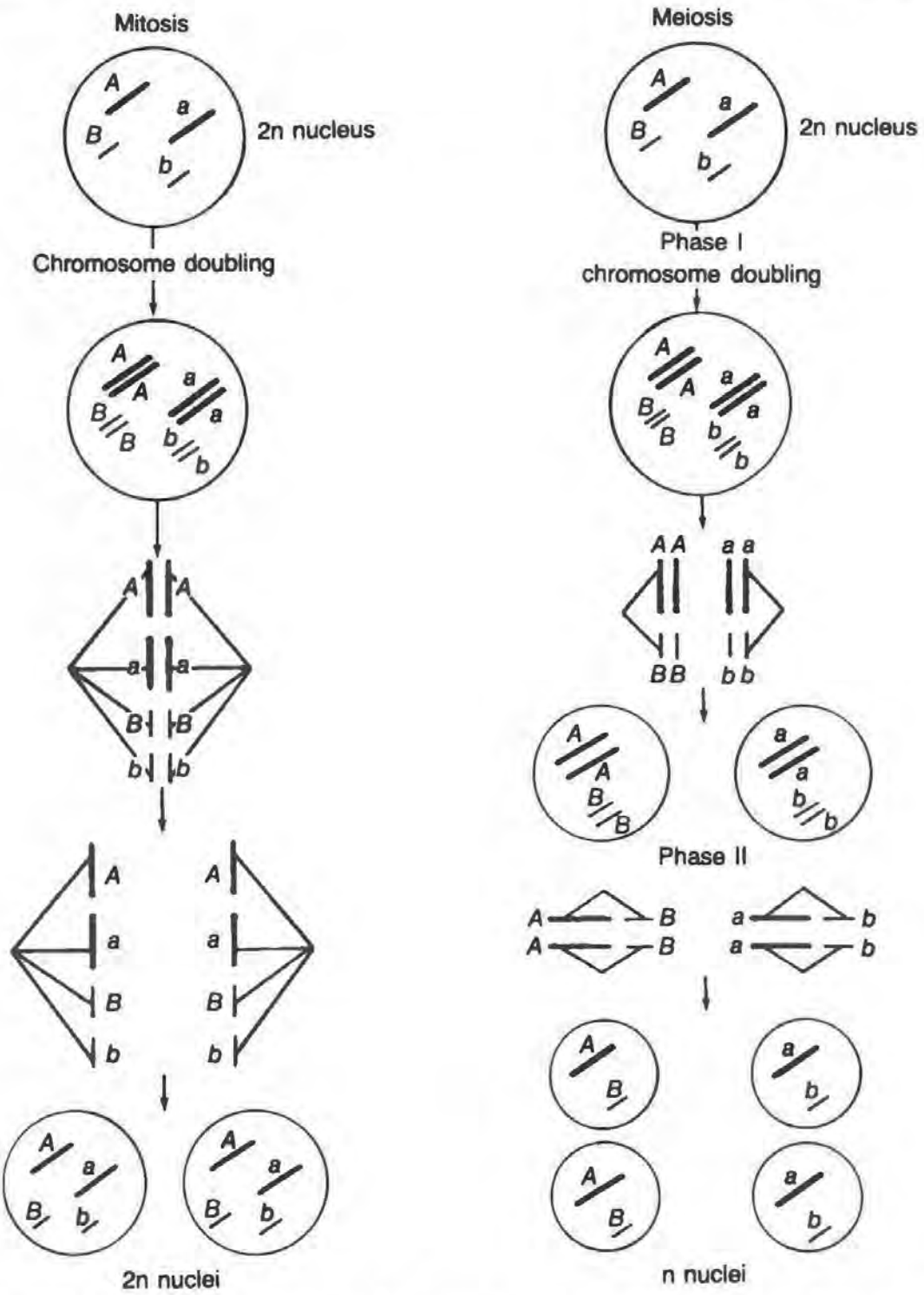


Figure 2–4 Mitosis results in the formation of unreduced (2n) daughter cells that are genetically identical to each other and to the parent cell (2n). Meiosis is reduction division of the 2n parent cell to produce four haploid (n) gametes. The random assortment of chromosomes during phase I of meiosis can result in haploid nuclei of the genotype *AB*, *aB*, *Ab*, or *ab*.

chromosomes uncoil until they resemble threadlike structures. A nuclear membrane forms around the chromosomes, and a cell wall develops between the two nuclei.

Mitosis

Mitosis is referred to as equational division, because the two cells produced by the process are genetically equivalent to the original mother cell (Fig. 2-4). The stages of mitosis are prophase, metaphase, anaphase, and telophase.

The DNA of each chromosome in the nucleus replicates to form two identical chromatids. The chromatids remain attached at the centromere region as the chromosomes coil and become thick during prophase. In contrast to the prophase of meiosis I, the prophase of mitosis does not involve pairing of chromosomes or crossing over between homologous chromosomes. During metaphase, the individual chromosomes line up at the equatorial plate of the cell and a spindle fiber develops that links each of their chromatids to one of the two poles in the cell. The chromatids of each chromosome separate and move to opposite poles at anaphase. At telophase, a nuclear membrane develops around the chromosomes to define the nucleus of the cell, and a cell wall is formed.

Gamete Formation and Fertilization

Each of the four microspores produced by meiosis develops into a pollen grain (Fig. 2–1). Each microspore undergoes a mitotic division to form a generative nucleus and a vegetative nucleus. The generative nucleus divides mitotically to produce two sperm cells.

Three of the four megaspores produced by meiosis disintegrate. The fourth megaspore undergoes three mitotic divisions to form the eight nuclei of the embryo sac (Fig. 2–1).

During fertilization, the egg cell in the embryo sac unites with one of the sperm cells from the pollen to form a 2n zygote. Mitotic division of the zygote results in the formation of the seed embryo. The two polar nuclei in the embryo sac unite with the second sperm cell from the pollen to form a 3n cell, which undergoes mitosis to form the endosperm of a seed.

SEXUAL REPRODUCTION

Seed production by sexual reproduction involves the transfer of pollen from an anther to the stigma of the pistil, movement of nuclei in the pollen tube through the style to the embryo sac, and union of functional male and female gametes. Seeds are classified according to the source of pollen that is responsible for fertilization. Self-pollinated seeds are formed when the pollen that effects fertilization is produced on the same plant as the female gamete with which it

unites. Cross-pollinated seeds result when the pollen of one plant is responsible for fertilization of the female gamete of another plant.

Plant species are classified according to the relative frequency of self-pollination and cross-pollination in their seed production. There is a continuum of variation among species, ranging from those with virtually complete self-pollination to those with only cross-pollination. For convenience, however, a species usually is designated as either self-pollinated (autogamous) or cross-pollinated (allogamous). The term "often cross-pollinated" is sometimes used to designate species with a more intermediate level of the two types of pollination.

The mechanism of pollination for a species is an important factor in determining the type of cultivar that is grown commercially and the breeding method used to develop the cultivar. Hybrid seed production is more readily accomplished in a cross-pollinated species than in a self-pollinated one. The formation of homozygous lines occurs naturally in self-pollinated species, but artificial self- or sib-pollination must be practiced in cross-pollinated species to obtain homozygous genotypes. (Chap. 8).

There are several natural mechanisms that favor self-pollination or cross-pollination. Some of the mechanisms can be readily overcome, while others are more difficult to manipulate.

Flower Morphology and Development

The type of flower that a species possesses contributes to the relative importance of self- and cross-pollination. The two structures directly involved in sexual reproduction are the male stamens and the female pistil. A stamen consists of an anther, which contains the pollen grains, and a filament on which the anther is borne. The number of anthers in a flower varies from 3 to 10 among most species.

The pistil includes an ovary, style, and stigma. The stigma is the receptor of pollen. Pollen germinates on the stigma and passes down the style and into the ovary. The ovary has one or more ovules, each containing an embryo sac (Fig 2-1). A 2n zygote is formed by the union of a sperm nucleus of the pollen with the egg cell of the embryo sac. The zygote divides mitotically to form the embryo of the seed. Union of a sperm nucleus with the two polar nuclei of the embryo sac results in a 3n cell that divides mitotically to form the endosperm of the seed.

The reproductive organs are enclosed in one or more protective structures. In some species, the enclosing structures are the calyx and the corolla. The calyx is composed of leaflike structures, referred to individually as sepals, and the corolla is made up of petals. The number of sepals in a calyx, the extent to which they are fused to each other, their size relative to the corolla, and their importance in enclosing the reproductive organs vary widely among species. Inside the calyx is the corolla, a collection of individual petals that often are the colorful portion of the flower. The individual petals may be similar or different

in size and shape and may be independent or fused. The corolla may be fused to or independent of the stamens.

In grass species, the reproductive organs of bisexual flowers may be enclosed by glumes, a lemma and palea, or both. The glumes may be large and enclose one or several flowers, or may be small structures that do not protect the reproductive organs. The lemma and palea surround the reproductive organs.

Flower morphology and development can influence self- and cross-pollination in several ways. The stamens and pistil may occur in the same or different flowers. Flowers with only one reproductive organ may occur on the same or different plants. The stamens and pistil may mature at the same or different times. Pollen shed may occur when the flower is open or closed. These variations and the terms associated with them have been summarized by Lersten (1980) as follows:

Floral characteristics	*Terms*
A. Male and female expression in individual flowers	
1. Male and female in one flower	Bisexual, hermaphroditic, monoclinous, perfect
a. Pollen shed before stigma is receptive	Protandry
b. Stigma matures and ceases to be receptive before pollen is shed	Protogyny
c. Stigma receptive, and pollen shed, after flower opens	Chasmogamy
d. Stigma receptive, and pollen shed, in closed flower	Cleistogamy
2. Perfect flowers of two types on same plant	
a. Long styles and short stamens	Pin flower
b. Short styles and long stamens	Thrum flower
3. Male and female in separate flowers	Diclinous, unisexual, imperfect
a. Male flower	Male, staminate
b. Female flower	Carpellate, female, pistillate
B. Flower distribution on plants	
1. Male and female flowers on one plant	Monoecious
2. Male and female flowers on separate plants	Dioecious
3. Male, female, and perfect flowers	Mixed, polygamous
a. On same plant	Polygamomonoecious
b. On separate plants	Polygamodioecious

Self-pollination is favored by pollen shed in a cleistogamous, perfect flower. Cross-pollination is favored by protandry, protogyny, chasmogamy, pin and thrum flowers, and imperfect flowers on monoecious, dioecious, or polygamous plants.

Cross-pollination can be enhanced in perfect flowers by physical barriers to self-pollination. For example, in alfalfa, a membrane over the stigma prevents self-pollen from effecting fertilization (Barnes, 1980). A bee carrying pollen lands on the alfalfa flower, which causes it to be tripped. The stigma strikes the bee, the stigmatic membrane is broken, and pollen from the bee's body is transferred to the stigma. The foreign pollen fertilizes the egg, resulting in cross-pollinated seeds.

Self-Incompatibility

Self-incompatibility is the inability of a plant with functional female and male gametes to produce a zygote by self-pollination. It is an effective mechanism for promoting cross-pollination in some plant species.

There are several mechanisms responsible for self-incompatibility in higher plants (Lersten, 1980): (a) Pollen may fail to germinate on the stigma. (b) Pollen tube growth in the style may be inhibited to the extent that pollen fails to reach the ovary. (c) Pollen tubes of sufficient length may fail to penetrate the ovule. (d) A male gamete that enters the embryo sac may fail to unite with the egg cell.

Gametophytic and Sporophytic Self-Incompatibility. Self-incompatibility is based on the genotypic and phenotypic relationship between the female and male reproductive organs. Alleles in cells of the pistil determine its receptivity to pollen. The phenotype of the pollen, expressed as its inability to effect fertilization, may be determined by its own alleles, referred to as gametophytic incompatibility, or by those of its maternal plant, referred to as sporophytic incompatibility. The two types of incompatibility will be compared for a diploid plant by assuming one locus (S) with multiple alleles, no dominance among alleles in an S_1S_3 female, and dominance in the male: $S_1 > S_2 > S_3 > S_4$.

A male gamete is able to effect fertilization in a species with gametophytic incompatibility if the allele it possesses at the S locus differs from both alleles in the pistil. With an S_1S_3 female, none of the gametes from an S_1S_3 male would be functional, but the S_2 gametes of an S_1S_2 or S_2S_3 male and the S_2 and S_4 gametes from an S_2S_4 male would be functional.

For a species with sporophytic incompatibility, all of the male gametes from a plant have the same ability to fertilize a female, regardless of their individual genotype. Under the dominance assumed in the illustration, an S_2 gamete of an S_1S_2 male would have the S_1 phenotype and an S_4 gamete from an S_2S_4 male would have the S_2 phenotype. With an S_1S_3 female, none of the gametes from

either S_1S_2 or S_1S_3 males would be able to effect fertilization; however, all of the gametes from an S_2S_3 or S_2S_4 male would be functional.

Homomorphic and Heteromorphic Floral Structure. The relative lengths of the stamens and style in a bisexual flower have been associated with incompatibility. Most species have homomorphic flowers in which the stamens and styles attain comparable lengths. Self-incompatibility in homomorphic flowers can be gametophytic or sporophytic.

A limited number of species exhibit heteromorphic flowers in which the stamens and styles attain different heights (Frankel and Galun, 1977). The presense of either pin or thrum flowers is termed distyly. Tristyly refers to three lengths of the style relative to the anthers: short, medium, or long. In a species with heteromorphic flowers, the inability to obtain self-pollinated seed is not caused by the structure of the flower, but by the presence of an associated sporophytic incompatibility system. Gametophytic incompatibility has not been found to be associated with heteromorphic flowers.

In species with distyly, pin × pin and thrum × thrum crosses are incompatible due to alleles at a single locus. The thrum morphology is controlled by the dominant allele *S* and the pin morphology by the recessive alleles. With a pin female (*ss*), none of the gametes from a pin male are functional, but both alleles from a thrum male (*Ss*) can effect fertilization. Thrum flowers are heterozygous because a thrum x thrum mating is not possible (Frankel and Galun, 1977).

The incompatible matings with tristyly are long × long, medium × medium, and short × short. The sporophytic incompatibility associated with style length is controlled by two loci, S and M, each with a dominant and recessive allele. Plants with long styles are homozygous recessive for both alleles, *ssmm*, and those with medium styles are *ssMm* or *ssMM*. The dominant allele *S* results in short styles regardless of the genotype at the M locus; therefore, plants with short styles may have the genotype *S__M__* or *S__mm*.

It should be emphasized that although sporophytic incompatibility and heteromorphism are closely associated in nature, there is good experimental evidence that incompatibility and floral morphology are controlled by distinct genetic components. It has been proposed that a complex gene controls distyly, consisting of five linked subunits (Nettancourt, 1977). The model indicates that two units control sporophytic incompatibility, one controls style length, one controls anther height, and one controls pollen size.

Partial Self-Incompatibility. Self-pollination in some species results in a lower percentage of seed set than occurs with cross-pollination. The production of some selfed seed indicates that the self-incompatibility is partial. There are quantitative differences among individuals for the percentage of seed set by self-pollination compared with that set by cross-pollination. The success of self-pollination ranges from a low level of seed set in some individuals to a high

level in others. Selection can be practiced for high or low levels of self-incompatibility within a species.

Partial self-incompatibility is associated with the lower effectiveness of self-pollen in causing fertilization compared with foreign pollen from a different individual. This characteristic permits the production of a high percentage of cross-pollinated seed by open-pollination, even though self-incompatibility is not complete.

Partial self-incompatibility is present in alfalfa (Barnes et al., 1972). Self-pollination of clones can reduce the percentage of flowers that set pods and the number of seeds per pod, compared with cross-pollination. The amount of reduction in seed set from self-pollination is highly variable among clones. Two mechanisms are responsible for the reduced ability of self-pollen to fertilize an ovule. First, pollen tubes from self-pollen often fail to grow long enough to reach the ovules most distant from the stigma. Second, pollen tubes of self-pollen that are long enough to reach an ovule do not penetrate it as frequently as pollen tubes of cross-pollen. In alfalfa, the percentage of fertilization occurring in ovules reached by pollen tubes was reported to be 28 percent for self-pollen and 80 percent for cross-pollen (Brink and Cooper, 1940a,b). Failure of ovule penetration seems to be the most important mechanism for self-incompatibility in this crop. The genetic basis of partial self-incompatibility systems has not been determined.

Male Sterility

Male sterility refers to the failure of a plant to produce functional pollen. Genetic and cytoplasmic-genetic systems that cause male sterility have been identified in several species. Male sterility is utilized by plant breeders to facilitate hybridization in breeding programs and for commercial production of hybrid seed. The utilization of male sterility in plant breeding is discussed further in Chap. 16 and 35.

ASEXUAL REPRODUCTION

Asexual propagation results in the multiplication of genetically identical individuals. An individual reproduced asexually is referred to as a clone, and the process by which it is multiplied is called cloning. For convenience, asexual reproduction will be divided into two aspects: (a) that occurring through plant parts other than seed and (b) that occurring through seed formed by apomixis.

Cloning Through Plant Parts Other than Seed

A number of different plant parts can be involved in the asexual reproduction of species.

Bulb. A bulb is a large bud with a small stem at its lower end. Numerous fleshy scalelike leaves grow from the upper surface and roots develop on the lower surface of the small stem. Buds in the axils of the scalelike leaves permit the bulb to be divided for asexual propagation, such as in the onion.

Corm. A corm resembles a bulb in size and form, but has a different internal structure. Its leaves are usually thin and small. Buds in the axils of the leaves permit asexual reproduction. Roots develop from the lower surface of the stem. Asexual reproduction by corm occurs in gladioli.

Rhizome. A rhizome is a horizontal stem that grows at or below the surface of the soil, such as occurs in *Iris* species. A rhizome can be mistaken for a root when it grows underground. A rhizome has nodes, internodes, buds, and often leaves. When a rhizome is cut from a plant and replaced in the soil, roots and stems develop from buds at the node.

Stolon. A stolon, or runner, is a stem that grows horizontally above the surface of the soil. Buds at the nodes of the stolon permit a clone to multiply asexually, such as in the strawberry and bermudagrass.

Tuber. A tuber is an enlarged underground stem. The "eyes" of a tuber, such as the potato, are buds from which stems and roots can develop to form new plants.

Cloning Through Seed Formed by Apomixis

The asexual propagation of plants by seed occurs in obligate and facultative apomicts. In obligate apomicts, all of the seed produced results from asexual reproduction, except for rare plants that are capable of some sexual reproduction. In facultative apomicts, most of the seed is produced asexually, but sexual reproduction regularly occurs. Apomixis is caused by several mechanisms that differ by the cell that undergoes mitosis to produce the embryo of the seed (Bashaw, 1980).

Apospory. Apospory occurs when somatic cells of the ovule divide mitotically to form a 2n embryo sac. The megaspore mother cell responsible for sexual reproduction in the ovule undergoes meiosis, but the megaspore or young embryo sac aborts. Apospory is the most common mechanism for apomixis in higher plants, such as buffelgrass and Kentucky bluegrass.

Diplospory. The origin of the embryo and endosperm of a seed produced by diplospory is the 2n megaspore mother cell. The nucleus of the megaspore mother cell undergoes mitosis, instead of meiosis, to form the embryo sac. Diplospory occurs in several genera of perennial grasses, including *Tripsacum*.

Adventitious Embryony. No embryo sac is formed during the development of seed by adventitious embryony. A 2n cell of the ovule, integuments, or ovary wall undergoes mitosis to form an embryo. The endosperm is assumed to arise from the polar nuclei of a normal embryo sac that develops independently in the ovule. This form of apomixis is common in *Citrus* species.

Parthenogenesis. Parthenogenesis is the development of a haploid embryo from the egg cell in a sexual embryo sac without fertilization by the sperm nucleus. The event probably occasionally occurs at random in most species. In addition, lines have been identified in some species in which parthenogenesis is under genetic control.

Androgenesis. In androgenesis, the embryo of a seed develops from the sperm nucleus of the pollen grain after it enters the embryo sac. The haploid plant that develops from the seed has the genetic makeup of the sperm. Androgenesis occasionally occurs at random in most species, but is under genetic control in some lines of maize (Kermicle, 1969).

Semigamy. Semigamy occurs when the sperm nucleus from the pollen enters the embryo sac and penetrates the egg cell, but the sperm nucleus and egg nucleus do not fuse to form a 2n zygote. Instead, the sperm and egg nuclei divide independently to form a haploid embryo. Haploid plants that develop from the embryo contain sectors of tissue of maternal or paternal origin. Semigamy has been reported in Pima cotton (Turcotte and Feaster, 1969).

Role of Pollination. Pseudogamy is the absolute requirement for pollination to obtain viable apomictic seed, even though the sperm nucleus and egg cell nucleus do not unite to form a zygote (Bashaw, 1980). Although its role is not fully understood, pollination seems to function as a stimulus for initiation of embryo and embryo sac development, for fertilization of the polar nuclei and endosperm development, or for full maturation of the embryo. Pseudogamy occurs in several economically important plants, including *Citrus* species, raspberry, apple, and some bluegrass species (Frankel and Galun, 1977).

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CHAPTER THREE

Genetic Principles

The phenotype of an individual is determined by its genotype and the influence of environment, expressed as phenotype = genotype + environment. Phenotype refers to the appearance or measurement of a character, such as red flowers, 500 g of seed, or 50 cm of height. Genotype refers to the genes that control a character. Environment includes all of the external factors that can influence the expression of the genes controlling a character, including moisture, soil fertility, temperature, and human actions.

Plant characters often are referred to as qualitative or quantitative, depending on the number of genes that control them and the importance of the environment in expression of the genes. Qualitative characters have phenotypes that can be divided into discrete classes (Fig. 3-1). They are controlled by one or a few major genes whose expression is not influenced markedly by the environment. Resistance to a disease is a qualitative character when it is controlled by a single gene and when resistant and susceptible plants can be clearly distinguished.

A quantitative character displays a continuous distribution of phenotypes (Fig. 3-1). The variability is associated with the segregation of multiple minor genes or polygenes, which have small individual effects and are influenced markedly by the environment. Seed yield is a quantitative character controlled by multiple genes and strongly influenced by environment.

Some plant characters exhibit aspects of both qualitative and quantitative inheritance (Fig. 3-1). These are characters that are controlled by one or a few major genes and by multiple genes with small effects. The genes with small effects, sometimes referred to as modifying genes, and the effect of the environment contribute to a phenotypic distribution that is continuous. The phenotypic distribution of segregates can have several modes, each of which represents the expression of a major gene. Plant height in maize generally is considered a quantitative character; however, it can be altered substantially by a single major gene, *brachytic-2*, that results in shortened internodes and reduced plant height

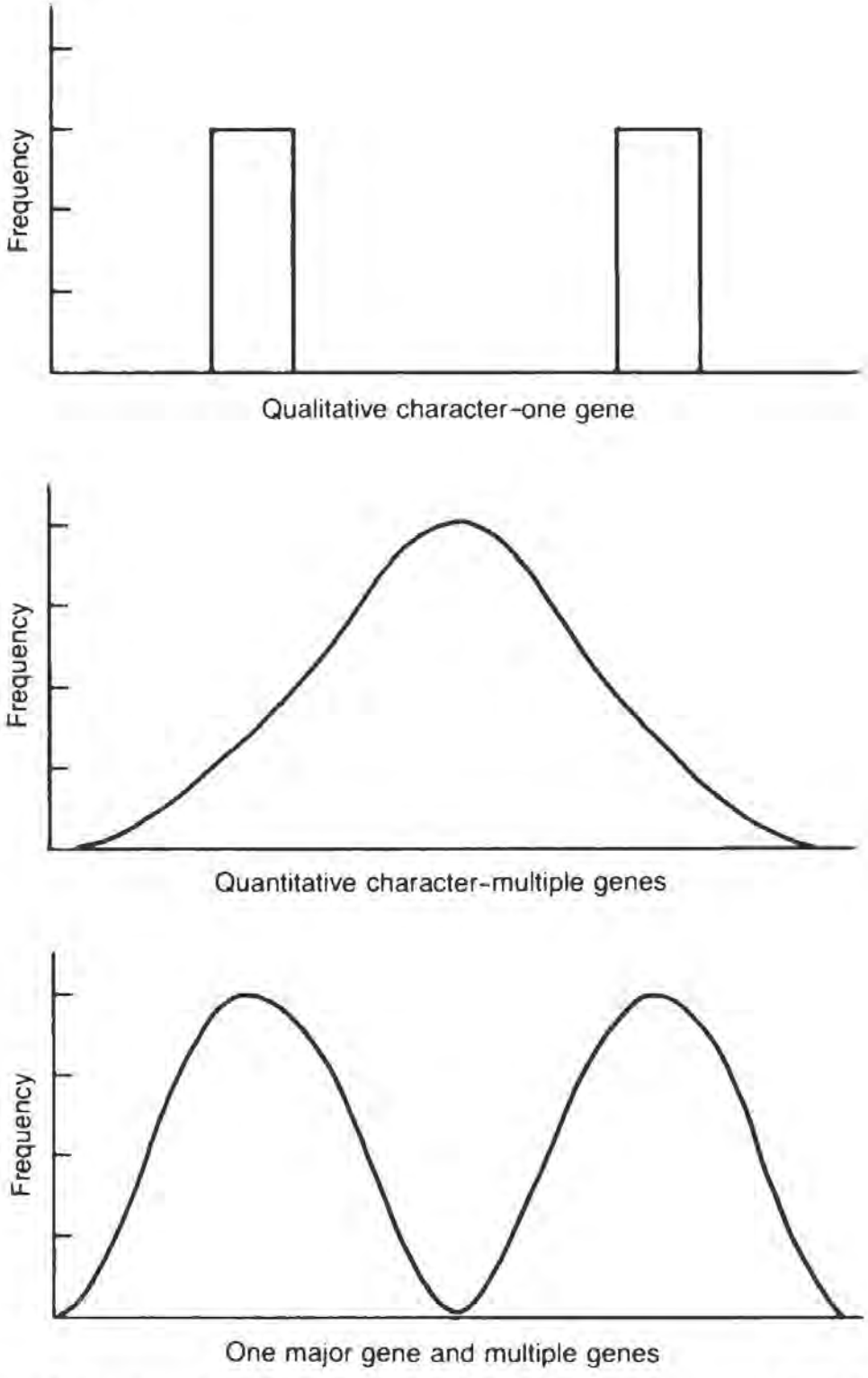


Figure 3–1 A qualitative character controlled by one gene can be divided into discrete phenotypic classes. A quantitative character controlled by many genes displays a normal distribution of phenotypes in a segregating population. Some characters are controlled by one or a few major genes and multiple genes with small effects.

(Pendleton and Seif, 1962). Time of maturity of soybean is controlled by several major genes and by multiple genes with small effects (Bernard and Weiss, 1973).

The genetic principles used by the plant breeder in the selection of qualitative and quantitative characters are based on the laws of heredity discovered by Gregor Mendel and later geneticists. An understanding of the genetic principles permits the breeder to select an appropriate breeding strategy for the character of interest. The principles associated with qualitative inheritance will be emphasized in this chapter. Aspects of quantitative inheritance are discussed in Chap. 6.

SYMBOLISM FOR DESCRIBING POPULATIONS AND INDIVIDUALS

Two symbols, F and S, are used by plant breeders to describe progeny developed by hybridization and self-pollination. F is derived from the word filial, which is defined as the sequence of generations after the mating of two parents. S is the symbol used to denote generations of self-pollination.

It is important to understand that there is more than one system for using the symbols F and S to describe populations, individuals, and inbred lines. For example, the S_0 generation for some breeders represents the hybrid plants obtained from the cross between two homozygous parents, also referred to as the F_1. Other breeders use the S_0 to designate the first segregating generation after the cross, also referred to as the F_2. The conversation between two breeders can be confusing and misleading if each has a different understanding of the genetic material represented by the symbol S or F.

Each of the systems used by plant breeders has advantages and disadvantages. It is not likely that breeders throughout the world will agree on a single system. Therefore, breeders should understand the different systems that are available and be able to detect which system is being used in writing or in conversation. Authors and speakers should clearly indicate the meaning of the symbols used in an article or speech.

F Symbol

F_1. The seed obtained from the cross between two parents is called the F_1 (Fig. 3-2). The genetic makeup of the F_1 is determined by the homozygosity of the parents. The single cross between two homozygous parents produces an F_1 population that is homogeneous because all of the F_1 plants are genetically identical. The F_1 plants are heterozygous for all loci at which there were different alleles in the two parents.

A heterogeneous population is produced whenever heterozygous parents are

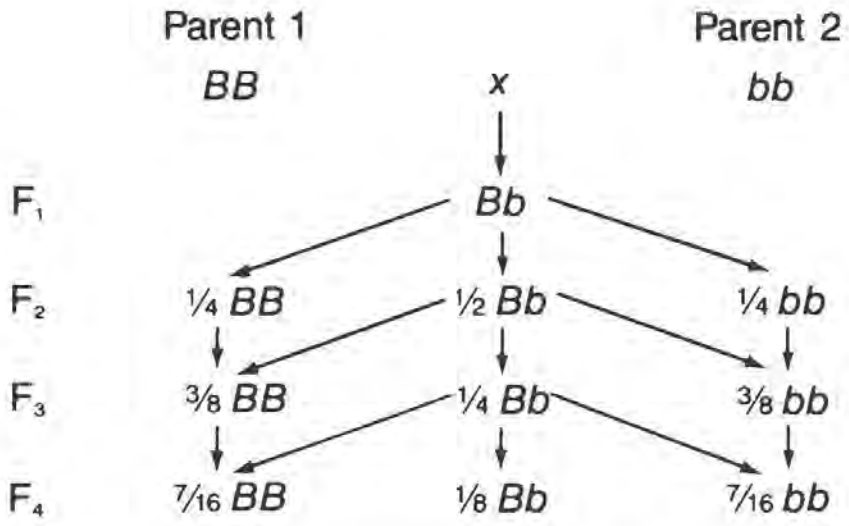


Figure 3–2 Segregation of a single gene in a diploid species. The fraction given for each genotype is the frequency expected on the average for each generation of self-pollination.

mated. One example is the mating of an F_1 from one single cross with an F_1 from another single cross to produce double-cross hybrid seed. Some breeders refer to this seed as F_1 seed, but it is considerably different genetically from the F_1 seed produced from the cross of two homozygous parents. The population of plants from a double cross is heterogeneous because the plants are genetically different. The genotypic frequency for any locus depends on the genotype of the four original parents.

Another example of a heterogeneous population developed by hybridization is the first generation offspring of a cross between two vegetatively propagated cultivars that are highly heterozygous. Some breeders refer to the hybrids as F_1 plants.

F_2. Self-pollination of a heterozygous diploid F_1 plant produces the F_2 generation in which the genotypic ratio for a single locus is 1 (homozygous for one allele):2 (heterozygous):1 (homogyzous for a second allele)(Fig. 3-2). The phenotypic ratio for a single gene depends on the degree of dominance exhibited.

The F_2 generation also can refer to the base population developed by multiple generations of random mating. The seed obtained by random mating is hybrid in the sense that it is produced by crossing plants artificially or naturally. The seed technically could be called F_1, but generally is not because the genotypic frequency in the population of hybrids obtained by random mating resembles an

F_2 from a single cross between two homozygous parents more than it resembles the F_1 of a single cross. Similarly, the heterogeneous hybrid seed obtained from the mating of two heterozygous cultivars is sometimes referred to as the F_2 generation instead of the F_1.

F_3 and Later F Generations. The most common usage of the symbols F_3, F_4, and later F generations is with respect to consecutive generations of self-pollination after the F_2. The genotypic frequency of the population can vary widely depending on such factors as the genetic composition of the F_2 population, population size, and the amount of selection that occurs during selfing.

The symbol F_3 and those for later F generations have been used to a limited extent in reference to a population that has undergone consecutive generations of random mating without selection. In a study of maize, Gardner and Lonnquist (1959) used the symbols in such a manner, as quoted in the following:

> The F_2 was produced by selfing F_1 plants and the F_8 was produced by allowing random pollination in large isolated blocks each generation. To advance from one generation to the next approximately 200 to 400 ears were chosen at random each year. Equal numbers of seeds were taken from each ear and composited to plant the next generation.

S Symbol

S_0. The symbol S_0 is used in two different ways to describe the offspring of a single cross between two homozygous parents. One system equates the S_0 with the F_1 generation, and the other system equates it with the F_2 generation. Those who prefer $S_0 = F_1$ indicate that the subscript 0 means that the plant was not developed by self-pollination. Breeders who prefer $S_0 = F_2$ indicate that in theoretical genetic studies, the reference or base population formed by random mating, which is heterogeneous and heterozygous, is designated as either S_0 or F_2, not as S_1.

S_1 and Later S Generations. The genotypic frequency in a population in the S_1, S_2, or later S generations depends on the system used to designate the S_0. The two possible relationships are

$S_0 = F_1$	$S_0 = F_2$
$S_1 = F_2$	$S_1 = F_3$
$S_2 = F_3$	$S_2 = F_4$
$S_3 = F_4$	$S_3 = F_5$

There can be considerable confusion and miscommunication between two breeders if, without knowing it, one uses $S_0 = F_1$ and the other uses $S_0 = F_2$.

SYMBOLISM FOR DESCRIBING INBRED LINES

Two systems are commonly used to describe the generation of inbreeding for lines derived from a population developed by hybridization. System I describes a line based on the generation of plants that are being grown at that moment. System II describes both the generation of the plant from which the line originated and the generation of plants that are being grown at that moment. Both systems can be used with the symbols F or S.

As an illustration of the two systems, let us consider a population in the F_2 generation. An F_2 plant is selected, and its F_3 seeds are planted the following season. The line would be referred to as

System I: F_3 line
System II: F_2-derived line in F_3 (for brevity in writing, it could be referred to as an $F_{2\cdot3}$ line)

The F_3 plants within the line are harvested together in bulk, and a sample of F_4 seeds are planted the following season. The line would be referred to as

System I: F_4 line
System II: F_2-derived line in F_4, or $F_{2\cdot4}$ line

A single F_4 plant is harvested from the line, and the F_5 seeds are planted in a row. The line would be referred to as

System I: F_5 line
System II: F_4-derived line in F_5, or $F_{4\cdot5}$ line

The two systems can be used in the same way with the symbol S. Consider a random-mated population on the S_0 generation. An S_0 plant is harvested and its S_1 seeds are planted. The line would be referred to as

System I: S_1 line
System II: S_0-derived line in S_1, or $S_{0\cdot1}$ line

An S_1 plant is harvested from the line and its S_2 seeds are planted. The line would be referred to as

System I: S_2 line
System II: S_1-derived line in S_2, or $S_{1\cdot2}$ line

S_2 plants in the line are self-pollinated, and their seeds are bulked. A sample of the S_3 seed is planted. The line would be referred to as

System I: S_3 line
System II: S_1-derived line in S_3, or $S_{1\cdot3}$ line

SYMBOLISM CHOSEN FOR THIS BOOK TO DESCRIBE POPULATIONS, INDIVIDUALS, AND LINES

The criterion used to select the symbolism for this book was that such symbolism must provide the clearest and most complete genetic information possible. The symbolism chosen will not be perfect in every detail because no perfect system exists. The patience of readers will be appreciated when the symbolism used in the book is not consistent with the system they are most familiar with.

F_1

F_1 will refer to the hybrids produced from the mating of homozygous parents. The F_1 generation will not be considered equivalent to the S_0 generation. A segregating population developed from the cross between two heterozygous parents will be considered the F_2 or S_0 generation, not the F_1.

$F_2 = S_0$

The first segregating generation produced from the cross of two or more parents will be considered the F_2 or S_0 generation. For homozygous parents, the F_2 or S_0 populations will be obtained by self-pollination of F_1 plants. The offspring obtained directly from the cross heterozygous parents will be designated F_2 or S_0 because they are the first segregating generation after the cross.

$F_3 = S_1$ and Later Generations

The F_3 or S_1 generations will represent the offspring from self-pollination of F_2 or S_0 plants. Subsequent generations also will be derived by self-pollination, and the F and S relationship will be

$$F_2 = S_0 \qquad F_4 = S_2$$
$$F_3 = S_1 \qquad F_5 = S_3$$

The offspring obtained by random mating of an F_2 population will not be considered F_3. In such cases, the initial segregating population will be designated Synthetic 1 (Syn 1), the offspring obtained by random mating of Syn 1 will be designated Syn 2, and random mating of Syn 2 will give rise to Syn 3.

Line Description

Lines will be described in this book by the method designated as System II, because this system provides more complete genetic information than does Sys-

tem I. For example, when breeders indicate that they are growing F_5 lines, it is not possible to know the expected genetic variability among or within the F_5 lines because they may have originated from F_2, F_3, or F_4 plants. If a breeder says the lines are F_2-derived in F_5, the expected genetic variability among and within lines can be determined readily.

System II also will be used to describe lines using the symbol S. For example, a line obtained by planting S_1 seed from an S_0 plant will be considered S_0-derived in S_1.

To save space in the book, an abbreviated form of System II will be used. Lines will be described with two subscripts separated by a colon. The digit preceding the colon will be the generation of the plant from which the line originated, i.e., the generation from which the line was derived. The digit after the colon will be the generation of the seeds or plants available at a particular time. Persons who prefer System I should note that the digit after the colon corresponds to the number that would be used in that system.

F_2-derived line in F_3 = $F_{2:3}$ line
F_2-derived line in F_8 = $F_{2:8}$ line
F_5-derived line in F_{12} = $F_{5:12}$ line
S_2-derived line in S_3 = $S_{2:3}$ line
S_3-derived line in S_7 = $S_{3:7}$ line

INHERITANCE OF A SINGLE GENE

Diploid plant species have two sets of chromosomes, each of which can possess a different allele of a gene. An individual is heterozygous (*Aa*) when two different alleles are present at a locus and is homozygous (*AA* or *aa*) when the same alleles are present on both chromosomes.

The alleles at a locus can interact in several ways, as measured by the phenotype of the heterozygote compared with that of the homozygotes (Table 3-1). The alleles act in an additive manner when performance of the heterozygote is intermediate to that of the homozygotes. An intermediate heterozygote reflects no dominance of one allele over the other. Partial dominance is present when

Table 3-1 Phenotypic Value in Arbitrary Units of Heterozygote Relative to Homozygotes for Different Levels of Dominance in a Diploid Individual

	Level of Dominance			
Genotype	Additive (No Dominance)	Partial Dominance	Complete Dominance	Overdominance
AA	5	5	5	5
Aa	3	4	5	6
aa	1	1	1	1

the heterozygote performance differs from the average performance of the homozygotes but is not equal to that of either homozygote. Complete dominance occurs when heterozygote performance equals that of one of the homozygotes. Overdominance is present when performance of the heterozygote exceeds the value of that of either homozygote.

Self-Pollination Without Selection

The segregation of a single gene associated with self-pollination can be illustrated by crossing two parents that are homozygous for different alleles of a single gene (Fig. 3-2). The F_1 seed obtains one set of chromosomes and one allele of each gene from each parent and is heterozygous (*Bb*). The F_1 plant produces haploid pollen grains and eggs with the two alleles (*B* and *b*) in a 1:1 ratio. During self-pollination, the pollen grains and eggs unite to produce the F_2 ratio of 1 *BB*:2 *Bb*:1 *bb*. Each generation of self-pollination, the homozygous plants produce only offspring like themselves, while the heterozygous plants produce offspring in the ratio of 1 *BB*:2 *Bb*:1 *bb*. As a result, self-pollination increases the frequency of the homozygous individuals and decreases the frequency of the heterozygotes.

The frequency of heterozygotes for a single gene in a population for any generation of self-pollination without selection can be determined by the formula $(1/2)^n$, where n is the number of generations of self-pollination. The frequency of heterozygotes in the F_2 is $(1/2)^1 = 1/2$, $F_3 = (1/2)^2 = 1/4$, and $F_5 = (1/2)^4 = 1/16$.

The frequency of both homozygous types is equal to $1 - (1/2)^n$. The frequency of both homozygotes in F_2 is $1 - (1/2)^1 = 1/2$, in F_3 is $1 - (1/2)^2 = 3/4$, and in F_5 is $1 - (1/2)^4 = 15/16$. The frequency of either homozygote is one-half the frequency of both homozygotes: $F_2 = (1/2)/2 = 1/4$, $F_3 = (3/4)/2 = 3/8$, $F_5 = (15/16)/2 = 15/32$.

The frequency of genotypes for a single gene in a self-pollinated population without selection can be computed readily without a formula (Fig. 3-2). The frequency of the heterozygote is written down for each generation beginning with the F_1 and continuing to the generation in question. The frequency of heterozygotes is reduced by 1/2 each generation of self-pollination: $F_1 = 1$, $F_2 = 1/2$, and $F_3 = 1/4$. The frequency of both homozygotes is the difference between 1 and the frequency of the heterozygotes in the generation being evaluated: $F_2 = 1 - 1/2 = 1/2$ and $F_3 = 1 - 1/4 = 3/4$.

The frequency or ratio of plants in a population with different genotypes, such as *BB*, *Bb*, and *bb*, is referred to as the genotypic frequency or ratio. The frequency or ratio of plants with different appearance (phenotype) is referred to as the phenotypic frequency or ratio. The phenotypic and genotypic frequencies are the same when there is partial dominance, overdominance, or no dominance, but differ when complete dominance is expressed.

Only the dominant and the recessive phenotypes are present when a character is controlled by a single gene with complete dominance. The frequency of the dominant class is the sum of the frequencies for the homozygous dominant and heterozygous individuals. The homozygous recessive individuals have a frequency of 1 minus the frequency of the dominant individuals. In a self-pollinated population without selection, the phenotypic ratio in the F_2 for a single gene is 1/4 *BB* + 1/2 *Bb* = 3/4 dominant and 1/4 *bb* = 1/4 recessive, and in the F_3 is 3/8 *BB* + 1/4 *Bb* = 5/8 dominant and 3/8 *bb* = 3/8 recessive.

Plants can be selected from a population at any generation of self-pollination. Progeny from a plant heterozygous for a single gene will segregate in the same manner as a population derived from an F_1 plant. The progeny from a heterozygous F_6 plant (*Bb*) will segregate in the ratio 1/4 *BB*:1/2 *Bb*:1/4 *bb*, the same as an F_2 population. If the progeny are self-pollinated and are advanced without selection to the next generation, they will segregate in the ratio 3/8 *BB*:1/4 *Bb*:3/8 *bb*, the same as an F_3 population.

The frequency of plants that will have segregating progeny depends on the frequency of heterozygous individuals in the generation of selection. The frequency of genotypes and phenotypes in the progeny of a heterozygous plant depends on the number of generations of self-pollination that have been conducted since the plant was selected. It is important to know, therefore, the generation of the progeny that are being grown and the generation in which the plant was selected to initiate the line.

The segregation among and within lines for a single gene can be illustrated with a hypothetical self-pollinated population segregating for disease resistance. Resistance is controlled by an allele *R* with complete dominance. Several questions can be asked about the population and lines derived from it.

1. *What is the frequency of susceptible plants in the self-pollinated population without selection in the F_2 and F_4 generations?*

   The answer is determined by the number of plants with the susceptible *rr* genotype. The frequency of heterozygotes in the population is reduced by one-half with each generation of self-pollination: $F_1 = 1$, $F_2 = 1/2$, $F_3 = 1/4$, and $F_4 = 1/8$. The frequency of both homozygotes is 1 minus the frequency of the heterozygote: $F_1 = 1 - 1 = 0$, $F_2 = 1 - 1/2 = 1/2$, $F_3 = 1 - 1/4 = 3/4$, and $F_4 = 1 - 1/8 = 7/8$. The frequency of only the homozygous recessive is one-half the frequency of both homozygotes: $F_2 = (1/2)/2 = 1/4$, $F_3 = (3/4)/2 = 3/8$, and $F_4 = (7/8)/2 = 7/16$. The answer to the question is that the frequency of susceptible plants (*rr*) is 1/4 in F_2 and 7/16 in F_4.

2. *What is the frequency of resistant plants in the self-pollinated population without selection in the F_2 and F_4 generations?*

   The frequency of resistant plants is the sum of the frequencies of *RR* and *Rr* plants. The procedure for determining the frequency of the genotypes in F_2

and F_4 is the same as used to answer question 1. The frequency in F_2 of *RR* 1/4 and of *Rr* is 1/2. Their sum of 3/4 is the frequency of resistant plants in F_2. The frequency in F_4 of *RR* is 7/16 and of *Rr* is 1/8. Their sum of 9/16 is the frequency of resistant plants in F_4.

3. *What frequency of $F_{2:3}$ lines would have segregating progeny?*

   The frequency of lines that segregate is equal to the frequency of heterozygous plants in the generation in which they were derived. The frequency of heterozygous plants (*Rr*) in F_2 is 1/2; therefore, the frequency of segregating $F_{2:3}$ lines is 1/2.

4. *What frequency of $F_{2:5}$ lines would have segregating progeny?*

   The frequency of heterozygous plants (*Rr*) in F_2 is 1/2; therefore, the frequency of segregating $F_{2:5}$ lines is 1/2.

5. *What is the frequency of susceptible plants in an $F_{2:3}$ line that is segregating for the R gene?*

   A heterozygous F_2 plant is equivalent genetically to an F_1; therefore, segregation among progeny from an F_1 is equivalent to segregation among F_3 progeny from a heterozygous F_2 (1/4 *RR*:1/2 *Rr*:1/4 *rr*). The frequency of susceptible plants (*rr*) in a segregating $F_{2:3}$ line is 1/4.

6. *What is the frequency of susceptible plants in an $F_{9:11}$ line that is segregating for the R gene?*

   A heterozygous F_9 plant is equivalent genetically to an F_1. The segregation among F_{10} progeny from an F_9 plant is equivalent to that among F_2 progeny of an F_1 plant (1/4 *rr* plants). Segregation among F_{11} progeny from an F_9 would be equivalent to that of F_3 progeny from an F_1 plant (3/8 *rr* plants). The frequency of susceptible plants in a segregating $F_{9:11}$ line is 3/8.

Self-Pollination with Selection

The genotypic and phenotypic frequencies for a single gene in a self-pollinated population can be altered by artificial or natural selection. When selection is completely effective for plants with the recessive genotype, the dominant allele will be completely eliminated from the population in one generation. Elimination of the recessive allele is more difficult, because this allele often is masked by the dominant allele in the heterozygote.

When selection is practiced for a dominant allele, the frequency of genotypes in the generation after selection is a function of the genotypic frequency in the generation of plant selection (Fig. 3-3). The frequency of recessive genotypes in the generation after selection can be determined by the following procedure.

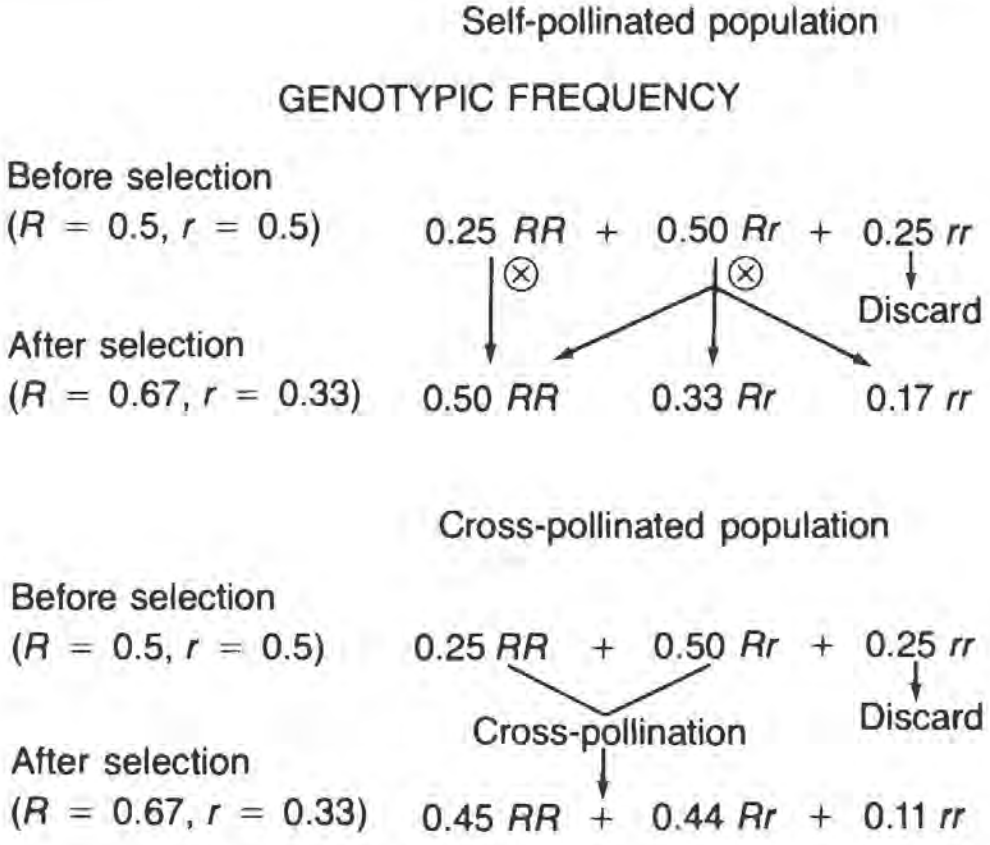


Figure 3–3 Effect of selection among plants before pollination for a dominant gene in a self-pollinated and in a cross-pollinated population.

Step 1: Compute the sum of the frequencies of homozygous dominant and heterozygous individuals in the generation in which selection is conducted.

$$F_2 = 1/4\ BB + 1/2\ Bb + 1/4\ bb$$

$$\text{Sum of } BB + Bb = 3/4$$

Step 2: Divide the frequency of homozygous dominant individuals before selection by the sum obtained in step 1 to obtain the frequency of homozygous dominant individuals after selection.

$$\frac{1/4\ BB}{3/4(BB + Bb)} = 1/3\ BB$$

Step 3: Divide the frequency of heterozygous individuals before selection by the sum obtained in step 1 to obtain the frequency of heterozygous individuals after selection.

$$\frac{1/2\ Bb}{3/4(BB + Bb)} = 2/3\ Bb$$

Step 4: The frequency of homozygous recessive individuals in the generation after selection is equal to one-fourth the frequency of heterozygous individuals after selection (step 3) because the heterozygotes segregate 1/4 *BB* + 1/2 *Bb* + 1/4 *bb*.

$$2/3\ Bb \times 1/4 = 1/6\ bb \text{ in } F_3$$

Step 5: The frequency of heterozygous individuals in the generation after selection is equal to one-half the frequency of heterozygous individuals (step 3) after selection.

$$2/3\ Bb \times 1/2 = 1/3\ Bb \text{ in } F_3$$

Step 6: The frequency of homozygous dominant individuals in the generation after selection is equal to the frequency of homozygous dominant individuals after selection plus one-fourth the frequency of heterozygous individuals after selection.

$$1/3\ BB + (2/3\ Bb \times 1/4) = 1/3 + 1/6 = 1/2\ BB \text{ in } F_3$$

The genotypic ratio of the F_3 population is *BB* (1/2) + *Bb* (1/3) + *bb* (1/6)

The following questions can be a posed to illustrate the effect of selection for a single gene controlling disease resistance and another controlling flower color in a self-pollinated population. Resistance is controlled by an allele *R* with complete dominance. White flowers (*pp*) are controlled by a recessive allele and purple flowers (*P_*) by an allele with complete dominance.

1. *What is the frequency of susceptible plants in the F_4 generation after selection for resistance among F_3 plants?*

Step 1: The genotypic frequency in the F_3 is

$$3/8\ RR + 1/4\ Rr + 3/8\ rr$$

The frequency of selected genotypes is 3/8 *RR* + 1/4 *Rr* = 5/8

Step 2: The frequency of *RR* after selection is

$$\frac{3/8\ RR}{5/8(RR + Rr)} = 3/5\ RR$$

Step 3: The frequency of *Rr* after selection is

$$\frac{1/4\ Rr}{5/8(RR + Rr)} = 2/5\ Rr$$

Step 4: The frequency of susceptible *rr* individuals in F_4 is

$$2/5\ Rr \times 1/4 = 1/10\ rr$$

2. *What is the frequency of white-flowered plants in the F_7 generation after selection for white-flowered plants in the F_6?*

The frequency is 1 because the recessive white-flowered plants in F_6 could have only white-flowered progeny in F_7.

3. *Susceptible plants are rogued from the population in the F_4 generation early in the season. At maturity, individual F_4 plants are harvested. What frequency of the $F_{4:5}$ lines would segregate for resistance?*

   Step 1: The genotypic frequency in the F_4 is

$$7/16\ RR + 1/8\ Rr + 7/16\ rr$$
$$RR + Rr = 9/16$$

   Step 2: Does not apply.

   Step 3: The frequency of segregating $F_{4:5}$ lines is equal to the frequency of heterozygous plants after selection in F_4.

$$\frac{1/8\ Rr}{9/16\ (RR + Rr)} = 2/9\ Rr$$

The frequency of segregating $F_{4:5}$ lines would be 2/9.

Cross-Pollination Without Selection

The genetic makeup of a population that is maintained by random cross-pollination varies with the frequency of the alleles that it possesses. The frequency of genotypes for a single gene with two alleles was defined for a diploid species by Hardy and Weinberg through independent research. When the gene frequencies and genotypic frequencies do not change from one generation to the next, a population is said to be in Hardy–Weinberg equilibrium. The genotypic frequency in a population at equilibrium is defined by the equation $p^2 + 2p(1 - p) + (1 - p)^2$, where p is the frequency of one allele and $(1 - p)$ is the frequency of the other. The sum of gene frequencies $p + (1 - p)$ is equal to one.

Assume that a single gene Y controls plant color in a cross-pollinated population of a diploid species. The frequency of Y is 0.3. What would be the frequency of YY, Yy, and yy in the population at Hardy–Weinberg equilibrium? The frequency of Y will be designated as p and the frequency of y as $(1 - p)$. p equals 0.3 and $1 - p$ equals 0.7. The frequency of genotypes is obtained by substituting frequencies of p and $(1 - p)$ in the equilibrium equation and calculating the solution.

$$p^2 + 2p(1 - p) + (1 - p)^2 = (0.3)^2 + 2(0.3)(0.7) + (0.7)^2 =$$
$$0.09\ YY + 0.42\ Yy + 0.49\ yy$$

The development of a population that is in Hardy–Weinberg equilibrium can be illustrated by the cross between two inbred parents that differ for alleles at one locus. The cross produces an F_1 hybrid that is heterozygous, and cross-

pollination among the hybrid plants produces a 1 *BB*:2 *Bb*:1 *bb* ratio, the expected ratio of a population at equilibrium. The criteria that must be met to reach and maintain a population at Hardy–Weinberg equilibrium are as follows.

1. There must be random mating in which each plant has an equal probability of being pollinated by every other plant, including itself. Many breeding populations are formed by mating 20 or fewer parents by artificial hybridization. Self-pollinations generally are excluded in such matings; therefore, Hardy–Weinberg equilibrium is closely approached but is not achieved. For example, assume that a population will be developed from four parents, two of which are *Bb* and two are *BB*. The gene frequency of *B* is equal to 0.75 and of *b* is equal to 0.25. The parents are mated in all combinations, excluding selfs and reciprocal crosses.

	Genotypes		
	BB	*Bb*	*bb*
Bb-1 × *Bb*-2 =	1	2	1
Bb-1 × *BB*-1 =	2	2	
Bb-1 × *BB*-2 =	2	2	
Bb-2 × *BB*-1 =	2	2	
Bb-2 × *BB*-2 =	2	2	
BB-1 × *BB*-2 =	4	—	—
Total	13	10	1
Genotypic frequency	0.54	0.42	0.04
Hardy–Weinberg equilibrium	0.56	0.38	0.06

The mating results in a population with genotypic frequencies that approach but do not achieve those that exist at equilibrium. To reach equilibrium, reciprocal crosses among the four parents and self-pollination of the parents must be included.

	Genotypes		
	BB	*Bb*	*bb*
Six single crosses in above table	13	10	1
Reciprocals of the six single crosses	13	10	1
Bb-1 self	1	2	1
Bb-2 self	1	2	1
BB-1 self	4		
BB-2 self	4		
Total	36	24	4
Genotypic frequency	0.56	0.38	0.06
Hardy–Weinberg equilibrium	0.56	0.38	0.06

2. No artificial or natural selection can occur within the population. The objective of plant breeding is to alter gene frequency in the desired direction; therefore, breeding populations are not expected to be in Hardy-Weinberg equilibrium for genes controlling characters under selection. Equilibrium may be maintained, however, for genes controlling independent characters that are not involved in selection.
3. Change by mutation should equally affect both alleles at a locus. Mutation tends to be more frequent from the dominant to the recessive form of the gene than vice versa; consequently, the criterion may not be met in a breeding population.
4. No loss of alleles or addition of alleles from an outside source should occur. Strict isolation of a cross-pollinated population would be required to meet this criterion.
5. Population sizes should be large enough that alleles are not excluded by genetic drift.

Failure to achieve Hardy–Weinberg equilibrium has no significant effect on cultivar development. The primary value of the concept is for understanding the effect of selection on gene and genotypic frequencies.

Cross-Pollination with Selection

The objective of selection in a cross-pollinated population is to change gene frequency in the desired direction. Selection for a recessive allele of a single major gene before pollination can eliminate the dominant allele in one generation. Assume that a population has a dominant allele *M* for multiple fruit in a frequency of 0.6 and an allele *m* for single fruit in a frequency of 0.4. The objective of selection is to select for the recessive allele. The genotypic frequency of the population at equilibrium is 0.36 *MM* + 0.48 *Mm* + 0.16 *mm*. Selection for the recessive allele before pollination would eliminate all the *MM* and *Mm* genotypes, and only the desired recessive allele would remain the next generation.

Selection for a dominant allele is hampered by masking of the recessive allele in the heterozygote (Fig. 3-3). The effect of selection on gene and genotypic frequency in an open-pollinated population depends on the timing of selection. Selection before pollination is twice as effective as selection after pollination.

Selection Before Pollination. The following procedure can be used to determine changes in gene and genotypic frequency with selection among plants in an open-pollinated population.

Step 1: Calculate the genotypic frequency in the population before selection with the equation $p_0^2 + 2p_0(1 - p_0) + (1 - p_0)^2$, where p_0 = frequency of the dominant allele and $1 - p_0$ = frequency of the recessive allele before selection.

Step 2: Eliminate the homozygous recessive individuals represented by the component $(1 - p_0)^2$, and add the frequencies of the homozygous dominant and heterozygous individuals: $p_0^2 + 2p_0(1 - p_0)$.

Step 3: Compute the frequency of the dominant allele after selection by using the relationship

$$p_1 = \frac{p_0^2 + p_0(1 - p_0)}{p_0^2 + 2p_0(1 - p_0)}$$

where p_1 = frequency of the dominant allele after one generation of selection.

Step 4: Calculate the frequency of the recessive allele after selection as $1 - p_1$ or as

$$1 - p_1 = \frac{p_0\,(1 - p_0)}{[p_0^2 + 2p_0(1 - p_0)]}.$$

Step 5: Compute the genotypic frequency in the population after selection as

$$p_1^2 + 2p_1(1 - p_1) + (1 - p_1)^2$$

The mathematical equations used to calculate the change in gene and genotypic frequency with selection in a random-mated population do not provide an understanding of the biological processes involved. The following example describes the five steps in biological terms, as well as mathematical terms. Assume that the dominant allele is *R* and the recessive allele is *r*.

Step 1: In a random-mated population, the sperm cell of every pollen grain has an equal chance of uniting with every egg cell. Assume that the frequency of alleles in the original population before selection is $R = 0.2$ and $r = 0.8$. If the original population were random-mated without selection, the gene frequencies would not change. Union of the sperm and egg cells can be diagrammed as

		Female	
		0.2 *R*	0.8 *r*
Male	0.2 *R*	0.04 *RR*	0.16 *Rr*
	0.8 *r*	0.16 *Rr*	0.64 *rr*

The genotypic frequency in the population would be $0.04\,RR + 0.32\,Rr + 0.64\,rr$. That is equivalent to $p^2 + 2p_0(1 - p_0) + (1 - p_0)^2 = (0.2)^2 + 2(0.2)(0.8) + (0.8)^2$.

If the only information available is the frequency of the dominant or recessive phenotype, the gene frequencies can be calculated from the recessive class. If the frequency of the recessive phenotype is 0.64, then $\sqrt{0.64} = 0.8\,r$ and $1 - 0.8 = 0.2\,R$.

Step 2: Elimination of the homozygous recessive individuals before pollination results in a population consisting of $0.04\ RR + 0.32\ Rr = 0.36$ of the original population.

Step 3: The homozygous dominant individuals produce gametes with the allele R, and half the gametes produced by the heterozygotes carry the R allele. The frequency of the R allele in the gametes available for fertilization would be

$$\frac{0.04\ RR + 1/2\ (0.32\ Rr)}{0.36\ (RR + Rr)} = 0.56\ R$$

This is equivalent to

$$p_1 = \frac{p_0^2 + p_0(1 - p_0)}{p_0^2 + 2p_0(1 - p_0)}$$

$$= \frac{(0.2)^2 + (0.2)(0.8)}{(0.2)^2 + 2(0.2)(0.8)} = 0.56\ R$$

Step 4: The gametes that do not have the R allele must have the r allele. Therefore, the frequency of r is $1 - 0.56\ R = 0.44$, which is equivalent to $1 - p_1$. An alternative procedure is to consider that half the gametes of the heterozygotes carry the r allele. The frequency of r can be calculated as

$$\frac{1/2\ (0.32\ Rr)}{0.36\ (RR + Rr)} = 0.44\ r$$

That is equivalent to

$$1 - p_1 = \frac{p_0(1 - p_0)}{p_0^2 + 2p_0\ (1 - p_0)} = \frac{0.2(0.8)}{(0.2)^2 + 2(0.2)(0.8)} = 0.44\ r$$

Step 5: The gametes produced by selected individuals would have the frequency of $0.56\ R$ and $0.44\ r$. Every gamete would have an equal chance to combine with every other gamete, which can be diagrammed as

		Female	
		0.56 *R*	0.44 *r*
Male	0.56	0.31 *RR*	0.25 *Rr*
	0.44	0.25 *Rr*	0.19 *rr*

The genotypic frequency in the new population would be $0.31\ RR + 0.50\ Rr + 0.19\ rr$. That is equivalent to

$$p_1^2 + 2p_1(1 - p_1) + (1 - p_1)^2 = (0.56)^2 + 2(0.56)(0.44) + (0.44)^2$$

Selection After Pollination. When selection is practiced before pollination, both the male and female parents are controlled, because the egg and sperm cells available for fertilization are from selected plants only. When selection occurs after pollination, only the female parent is controlled: the eggs available for fertilization are from selected plants but the pollen grains come from both selected and unselected plants. As a result, selection after pollination is only half as effective in changing gene frequency as selection before pollination.

The procedure for calculating the change in gene and genotypic frequency with selection after pollination is the same as that described for selection before pollination, except for step 5.

Steps 1 to 4: Calculation of the frequency of the dominant and recessive allele applies only to the female gametes. There is no selection of the male parent; therefore, there is no change in the frequency of alleles among male gametes.

Step 5: The frequency of alleles from the selected female parent is equal to p_1 and $1 - p_1$, while the frequency from the unselected male parent is p_0 and $1 - p_0$. The genotypic frequency in the next generation can be calculated as

$$p_0p_1 + [p_0(1 - p_1) + p_1(1 - p_0)] + (1 - p_0)(1 - p_1)$$

Assume, as with the example used for selection before pollination, that the frequency of $R = p_0 = 0.2$ and of $r = 1 - p_0 = 0.8$ in the original population. With selection after pollination, the gene frequency for the selected female becomes $R = p_1 = 0.56$ and $r = 1 - p_1 = 0.44$. The gene frequency for the unselected male remains at $R = p_0 = 0.2$ and $r = 1 - p_0 = 0.8$. The union of gametes to form the new population can be diagrammed as

		Female	
		0.56 *R*	0.44 *r*
Male	0.2 *R*	0.11 *RR*	0.09 *Rr*
	0.8 *r*	0.45 *Rr*	0.35 *rr*

The genotypic frequency is 0.11 *RR* + 0.54 *Rr* + 0.35 *rr*. That is equivalent to

$$p_0p_1 + [p_0(1 - p_1) + p_1(1 - p_0)] + (1 - p_0)(1 - p_1) =$$
$$(0.2)\ (0.56) + [(0.2)(0.44) + 0.56(0.8)] + 0.8\ (0.44)$$

Comparison of Selection Before and After Pollination. In the preceding example, the genotypic frequency of the original population was 0.04 *RR* + 0.32 *Rr* + 0.64 *rr* and the gene frequency was $R = 0.2$ and $r = 0.8$. With selection before pollination, the new population had a genotypic frequency of 0.31 *RR* + 0.5 *Rr* + 0.19 *rr* and a gene frequency of 0.56 *R* and 0.44 *r*. With selection after

pollination, the new population had a genotypic frequency of 0.11 *RR* + 0.54 *Rr* + 0.35 *rr* and a gene frequency of 0.38 *R* and 0.62 *r*.

This example illustrates an important plant-breeding principle: that selection before pollination in an open-pollinated population is twice as effective in changing gene frequency as selection after pollination.

	Gene Frequency	
	R	*r*
Original population	0.2	0.8
Selection after pollination	0.38	0.62
Selection before pollination	0.56	0.44

This principle will be an important consideration in recurrent selection (Chap. 15) and in maximizing genetic improvement (Chap. 17). In these two chapters, parental control will be discussed for various methods of recurrent selection in open-pollinated populations. Selection after pollination will be referred to as selection of the female only, and the parental control will be one-half (half as effective as selection before pollination). Selection before pollination will be referred to as selection of both parents, and the parental control will be one.

Effect of Selection in Self- Versus Cross-Pollinated Populations

The outcome of selection among plants in a self-pollinated population is the same as that for selection before pollination in a cross-pollinated population when selection is for the recessive phenotype. If plants can be classified without error, selection of the recessive phenotype will eliminate the dominant allele in one generation, regardless of the mode of pollination.

The effect of selection is not the same when selection is for the dominant allele. A gamete in a self-pollinated population is restricted to mating with a gamete of the same plant, whereas a gamete in a cross-pollinated crop has the possibility of mating with a gamete from any plant. Figure 3-3 illustrates the effect of selection before pollination for a dominant allele in self-pollinated and cross-pollinated populations. Note that the homozygous-dominant individuals in a self-pollinated population will only bear seed that is homozygous dominant. In contrast, the homozygous-dominant individuals in a cross-pollinated population receive pollen containing either the dominant or recessive allele. As a result, some of the seed in homozygous plants will be heterozygous.

This difference between self- and cross-pollinated populations is important to consider when discussing breeding methods. For example, the term mass selection is used by breeders of both self- and cross-pollinated species. Mass selection refers to selection among individual plants or seeds followed by bulking

together selected individuals to form a new population. The effect of selection is different for the two modes of reproduction and for the timing of selection relative to pollination in a cross-pollinated population. For that reason, breeders should carefully describe in both writing and verbal communication what is meant by the term mass selection. Some persons have chosen to clarify the meaning by referring to mass selection with self-pollination when a self-pollinated population is involved, and to mass selection with cross-pollination or recurrent phenotypic selection when a cross-pollinated population is involved.

INHERITANCE OF TWO OR MORE GENES

Independent Characters

A superior cultivar may require two characters, each controlled by single genes that are inherited independently. The characters may include resistance to two different diseases or resistance to a disease and an insect.

In the cross between two inbred parents that differ by alleles at two loci, the F_1 will be heterozygous for both loci (Fig. 3-4). Independent segregation at the two loci will produce four gametes in equal frequency. With self-pollination of the F_1, the four types of male gametes combine with those of the female in all combinations to form nine genotypes. The frequency of some genotypes is greater than that of others because they are formed by two or more different combinations of gametes. The heterozygote *AaBb*, which occurs in one-fourth of the genotypes in the F_2, can be formed by union of the gametes $AB + ab$, $Ab + aB$, $aB + Ab$, and $ab + AB$. The homozygote *aabb* with an F_2 frequency of 1/16 can be formed only by the union of identical gametes from both the male and female, $ab + ab$.

The frequency of phenotypes in the F_2 for the two characters considered together will depend on the level of dominance expressed by the two genes. The phenotypic and genotypic frequencies are the same when the heterozygotes for both characters express no dominance or partial dominance. Three phenotypes are expressed for each character, resulting in nine phenotypes when both characters are considered jointly. The phenotypic and genotypic frequencies differ when one or both heterozygotes for the two characters express complete dominance. No dominance or partial dominance for one character and complete dominance for the other results in a phenotypic ratio of 6:3:3:2:1:1. Complete dominance for both characters produces a phenotypic ratio of 9:3:3:1.

Self-pollination of the population to the F_3 and later generations without selection increases the frequency of the homozygous loci for both characters (Fig. 3-4). The phenotypic frequency changes in relation to the changes in the genotypic frequency.

Number of Genotypes and Phenotypes. The number of genotypes present when two or more independent genes are considered together can be calculated from

Parent 1 *AAbb* × *aaBB* Parent 2

↓

F_1 *AaBb*

↓

F_2

♀

♂	*AB*	*Ab*	*aB*	*ab*
AB	*AABB*	*AABb*	*AaBB*	*AaBb*
Ab	*AABb*	*AAbb*	*AaBb*	*Aabb*
aB	*AaBB*	*AaBb*	*aaBB*	*aaBb*
ab	*AaBb*	*Aabb*	*aaBb*	*aabb*

	Phenotypic frequency		
Genotypic frequency	*Aa*-pink *Bb*-intermediate	*Aa*-pink *Bb*-resistant	*Aa*-red *Bb*-resistant
1 *AABB*	1-red, resistant	1	9 *A_B_* red, res.
2 *AABb*	2-red, intermediate	2 ├ red, res.	
1 *AAbb*	1-red, susceptible	1-red, susc.	
2 *AaBB*	2-pink, resistant	2	3 *A_bb* red, susc.
4 *AaBb*	4-pink, intermediate	4 ├ pink, res.	
2 *Aabb*	2-pink, susceptible	2-pink, susc.	3 *aaB_* white, res.
1 *aaBB*	1-white, resistant	1	
2 *aaBb*	2-white, intermediate	2 ├ white, res.	
1 *aabb*	1-white, susceptible	1-white, susc.	1 *aabb* white, susc.

F_2	⊗ → F_3
1 *AABB*	9 *AABB*
2 *AABb*	6 *AABb*
1 *AAbb*	9 *AAbb*
2 *AaBB*	6 *AaBB*
4 *AaBb*	4 *AaBb*
2 *Aabb*	6 *Aabb*
1 *aaBB*	9 *aaBB*
2 *aaBb*	6 *aaBb*
1 *aabb*	9 *aabb*

Figure 3–4 Segregation of two independent genes. For the phenotypic ratios in F_2, the pink phenotype for *Aa* and intermediate resistance for *Bb* represent no or partial dominance. The red phenotype for *Aa* and resistant phenotype for *Bb* represent complete dominance.

the expression 3^n, where n = the number of segregating genes. For two genes ($n = 2$), there are $3^2 = 9$ genotypes, for three genes $3^3 = 27$, and for six genes $3^6 = 729$ genotypes.

The number of phenotypes present is determined by multiplying together the number of phenotypes expressed for each character. There are three phenotypes for a character with no or partial dominance and two phenotypes for characters with complete dominance. When three characters are considered, two with no dominance and one with complete dominance, the number of phenotypes is $3 \times 3 \times 2 = 18$. If the three characters have complete dominance, the number of phenotypes is $2 \times 2 \times 2 = 8$. When all characters have complete dominance, the number of phenotypes can be determined from the expression 2^n, where n = the number of segregating loci.

Calculation of Genotypic and Phenotypic Ratios. There are two alternatives for determining the genotypic and phenotypic ratios for two or more independent characters. The alternative used to clearly understand the biological basis of the genotypic ratio involves the use of a square in which the gametes produced by an individual are identified by columns and rows for the male and female, and the genotypes of the zygotes are written in the center (Fig. 3-4). The number of each genotype can be readily counted to obtain the genotypic frequency. The phenotypic frequency is derived directly from the genotypic frequency by identifying genotypes with a common phenotype and adding their frequencies.

The second alternative is to calculate the genotypic or phenotypic frequencies by multiplying the independent frequencies of each genotype or phenotype for the characters of interest.

F_2. In the F_2, the genotypic frequency of each homozygote for a single gene is 1/4 and the frequency of the heterozygote is 1/2 = 2/4. For independent genes, the genotypic frequency of *AABB* = 1/4 × 1/4 = 1/16, of *AaBB* = 2/4 × 1/4 = 2/16, and of *AaBb* = 2/4 × 2/4 = 4/16. The genotypic frequency for more than two independent genes is calculated in the same manner, *AABBccDD* = 1/4 × 1/4 × 1/4 × 1/4 = 1/256, *AaBbCcDd* = 2/4 × 2/4 × 2/4 × 2/4 = 16/256.

The phenotypic frequency in the F_2 depends on the dominance expressed by the heterozygote. With no dominance or with partial dominance for a character, the homozygotes each have a frequency of 1/4 and the heterozygote a frequency of 2/4. In the example in Fig. 3-4, *AABB* = 1/4 × 1/4 = 1/16 red, resistant; *AaBB* = 2/4 × 1/4 = 2/16 pink, resistant; and *AaBb* = 2/4 × 2/4 = 4/16 pink, intermediate. With complete dominance for a gene, the frequency of the dominant phenotype is 3/4, the sum of the frequencies of the dominant homozygote and heterozygote (1/4 *BB* + 1/2 *Bb* = 3/4 *B_*). The recessive phenotype has a frequency of 1/4. With one gene expressing no dominance (*Aa*) and the other complete dominance (*Bb*), the phenotypic frequency of *AAB__* is 1/4 × 3/4 = 3/16 red, resistant; of *AaB__* is 1/4 × 3/4 = pink, resistant; and of *aabb* is 1/4 × 1/4 = 1/16 white, susceptible. When both genes express complete dominance,

the phenotypic frequency of $A\_\_B\_\_$ is 3/4 × 3/4 = 9/16 red, resistant; of $A\_\_bb$ is 3/4 × 1/4 = 3/16 red, susceptible; and of *aabb* is 1/4 × 1/4 = 1/16 white, susceptible. The calculations of phenotypic frequencies for more than two genes involves multiplying the independent frequencies of the phenotypes for the characters. With complete dominance at four loci, the frequency of the dominant phenotype for all loci is $A\_\_B\_\_C\_\_D\_\_$ = 3/4 × 3/4 × 3/4 × 3/4 = 81/256.

F_3 and Later F Generations. The frequency of any genotype or phenotype can be determined for any generation of self-pollination. In the F_3, the frequency of each homozygote is 3/8 and of the heterozygote is 1/4 = 2/8. The frequency of the genotype *AABBCC* is 3/8 × 3/8 × 3/8 = 27/512; of *AaBBCC* is 2/8 × 3/8 × 3/8 = 18/512; and of *AaBbCc* is 2/8 × 2/8 × 2/8 = 8/512. In the F_4, the frequency of each homozygote is 7/16 and of the heterozygote is 1/8 = 2/16. The frequency of the genotype *AABBCC* is 7/16 × 7/16 × 7/16 = 343/4096, of *AaBBcc* is 2/16 × 7/16 × 7/16 = 98/4096, and of *AaBbCc* is 2/16 × 2/16 × 2/16 = 8/4096.

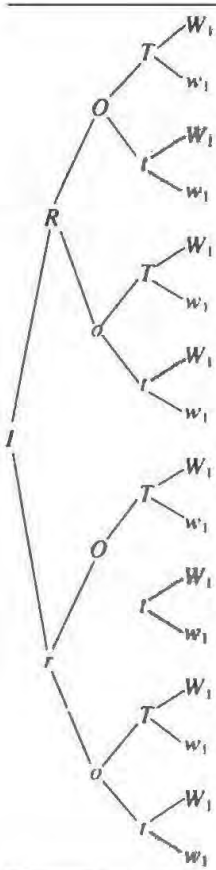
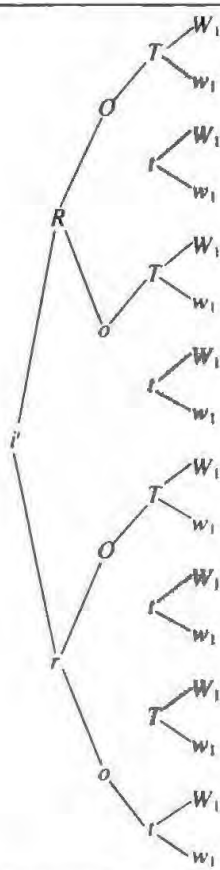
The phenotypic frequencies in each generation of self-pollination are calculated from the independent frequencies of the phenotypes of each character. In the F_4, a character with no dominance would have 7/16 of the phenotypes associated with each homozygote and 2/16 of the phenotype of the heterozygote. For complete dominance, the dominant phenotype would have the frequency of the homozygote plus that of the heterozygote, 7/16 + 2/16 = 9/16, and the recessive phenotype would have the frequency of the homozygote, 7/16. When three loci express complete dominance in the F_4, the frequency of individuals with the dominant phenotype for all characters would be $A\_\_B\_\_C\_\_$ = 9/16 × 9/16 × 9/16 = 729/4096.

Frequency of Homozygous Individuals. The frequency of individuals in a population that are homozygous for all genes of interest may influence the number of generations of self-pollination that are conducted before selection is initiated. When all homozygous individuals are considered regardless of the allele present at a locus, their frequency in the population can be expressed as $[(2^m - 1)/2^m]^n$, where m is the number of selfing generations (F_2 = 1, F_3 = 2) and n is the number of segregating loci. For an F_3 population with three segregating loci, the frequency of homozygous individuals would be $[(2^2 - 1)/2^2]^3$ = 27/64.

Interaction of Genes Controlling One Character

A character can be controlled by genes that are inherited independently but interact to form one phenotype. The hilum color of soybean seed involves the interaction of five genes, one of which controls pubescence color (T,t) and another that controls flower color (W_1, w_1) (Table 3-2). The interaction of genes at different loci that affect the same character is called epistasis. Epistasis was originally used by Bateson in 1909 to describe two different genes that affect

Table 3-2 Interaction of Genes Controlling Hilum Color in Soybean Seed*

	Hilum Color	Pubescence Color	Flower Color		Hilum Color	Pubescence Color	Flower Color
	Gray	Tawny	Purple		Black	Tawny	Purple
	Gray	Tawny	White		Black	Tawny	White
	Gray	Gray	Purple		Imperfect black	Gray	Purple
	Yellow	Gray	White		Buff	Gray	White
	Gray	Tawny	Purple		Black	Tawny	Purple
	Gray	Tawny	White		Black	Tawny	White
	Gray	Gray	Purple		Imperfect black	Gray	Purple
	Yellow	Gray	White		Buff	Gray	White
	Yellow	Tawny	Purple		Brown	Tawny	Purple
	Yellow	Tawny	White		Brown	Tawny	White
	Yellow	Gray	Purple		Buff	Gray	Purple
	Yellow	Gray	White		Buff	Gray	White
	Yellow	Tawny	Purple		Reddish brown	Tawny	Purple
	Yellow	Tawny	White		Reddish brown	Tawny	White
	Yellow	Gray	Purple		Buff	Gray	Purple
	Yellow	Gray	White		Buff	Gray	White

*The genes *I*, *R*,and *O* control the distribution and color of pigmentation in the seed. The genes for pubescence (*T*,*t*) and flower color (W_1,w_1) also are part of the genetic system controlling hilum color. To determine the genes controlling a particular hilum color, begin with W_1 or w_1 and follow the line back to *I* or i^i. Several different gene combinations can result in the same hilum color. (Adapted from R. G. Palmer, personal communication.)

the same character, one of which masks the expression of the other. The gene that masks another is said to be epistatic to it, and the gene whose expression is masked is hypostatic. In the example of hilum color of soybean, expression of the W_1, T, and O alleles is masked by the recessive allele r in the presence of I to produce yellow hilum color (Table 3-2). Said another way, the genotype $I\_\_rr$ will produce a yellow hilum regardless of the genotype at the O, T, and W_1 loci. The meaning of epistasis has been broadened to embrace all forms of interaction between loci, including cases in which neither locus is dominant to the other. Epistasis also is referred to as intergenic or interallelic interaction.

Epistasis causes deviations from the common phenotypic ratio in F_2 of 9:3:3:1 that is associated with segregation of two independent genes, each with complete dominance, which do not interact. Alternative forms of epistasis are summarized in Table 3-3.

Table 3-3 Phenotypic Ratios in F_2 for Two Unlinked Genes as Influenced by Degree of Dominance at Each Locus and Epistasis Between Loci

F_2 Genotypes									Genetic Basis of Ratio*
AABB	*AABb*	*AaBB*	*AaBb*	*AAbb*	*Aabb*	*aaBB*	*aaBb*	*aabb*	
1	2	2	4	1	2	1	2	1	Complete dominance lacking at either locus, no epistasis. Phenotypic and genotypic ratios equal.
3		6		1	2	3		1	Complete dominance lacking in *A*. Complete dominance of *B*. No epistasis.
9				3		3		1	Complete dominance in *A* and *B*. No epistasis.
9				3		4			Recessive epistasis. *aa* epistatic to *B,b*.
12						3		1	Dominant epistasis. *A* epistatic to *B,b*.
13						3			Dominant and recessive epistasis. *A* epistatic to *B,b*; *bb* epistatic to *A,a*. *A* and *bb* produce identical phenotype.
9				7					Duplicate recessive epistasis. *aa* epistatic to *B,b*; *bb* epistatic to *A,a*.
15								1	Duplicate dominant epistasis. *A* epistatic to *B,b*; *B* epistatic to *A,a*.
9				6				1	Duplicate genes with interaction. *A__bb* and *aaB__* have identical phenotype. *A__B__* and *aabb* have different phenotypes.

*For all cases of epistasis presented, it is assumed that there is complete dominance of *A* and *B* when the two loci are considered separately.

Source: Adapted from Snyder and David, 1957.

Recessive Epistasis—9:3:4 Ratio. Recessive epistasis occurs when recessive alleles at one locus mask the expression of both alleles at another locus. Assume that allele *a* is recessive to allele *A* but is epistatic to alleles *B* and *b*. *A__B__* individuals, which are 9/16 of the F_2, have one phenotype and *A__bb* genotypes (3/16) have a second phenotype. The *a* allele in the homozygous condition produces a third phenotype from *aaB__* (3/16) + *aabb* (1/16) individuals.

Dominant Epistasis—12:3:1 Ratio. A dominant allele at one locus can mask the expression of both alleles at another locus. Assume the *A* allele is dominant to *a* and epistatic to alleles *B* and *b*. *A__B__* (9/16) + *A__bb* (3/16) individuals in the F_2 have one phenotype, *aaB__* (3/16) produces a second phenotype, and *aabb* (1/16) results in a third phenotype.

Dominant and Recessive Epistasis—13:3 Ratio. A dominant allele at one locus can mask the expression of both alleles at a second locus, while a recessive allele at the second locus masks expression of alleles at the first locus. Assume that allele *A* is epistatic to alleles *B* and *b*, and *b* is epistatic to *A* and *a*. One phenotype is produced in F_2 by *A__B__* (9/16) + *A__bb* (3/16) + *aabb* (1/16) individuals and a second phenotype is produced by *aaB__* (3/16) individuals.

Duplicate Recessive Epistasis—9:7 Ratio. This form of interallelic interaction also is referred to as complementary epistasis. Recessive alleles at either of two loci can mask the expression of dominant alleles at the two loci. Assume that allele *a* is epistatic to alleles *B* and *b*, and *b* is epistatic to *A* and *a*. *A__B__* (9/16) individuals in F_2 have one phenotype and *aaB__* (3/16) + *A__bb* (3/16) + *aabb* (1/16) individuals have a second phenotype.

Duplicate Dominant Epistasis—15:1 Ratio. Dominant alleles at either of two loci can mask the expression of recessive alleles at the two loci. Assume that allele *A* is epistatic to alleles *B* and *b*, and *B* is epistatic to *A* and *a*. One phenotype is produced in F_2 by *A__B__* (9/16) + *A__bb* (3/16) + *aaB__* (3/16) individuals and a second phenotype results from *aabb* (1/16) individuals.

Duplicate Genes with Interaction—9:6:1 Ratio. A 9:6:1 ratio can occur when *A__B__* (9/16) individuals have one phenotype, *A__bb* (3/16) + *aaB__* (3/16) individuals have a second phenotype, and *aabb* (1/16) individuals produce a third phenotype.

Linkage of Genes

Genes on the same chromosome are linked if they do not segregate independently. Two heterozygous loci may be linked in coupling or repulsion. Coupling is present when the dominant or favorable alleles at two loci are on one chromosome and the recessive or unfavorable alleles are on the other, *AB/ab*. Repulsion is

present when a dominant or favorable allele at one locus is on the same chromosome as a recessive or unfavorable allele at another locus, *Ab/aB*.

The degree of linkage is described by the number of crossover or recombination units between two loci. Procedures for evaluation of linkage have been summarized by Mather (1951). One procedure is to cross an individual that is heterozygous at two loci (*AaBb*) to a homozygous recessive individual (*aabb*). If the two loci are not linked, the genotypic frequency of the progeny should average 1/4 *AaBb*, 1/4 *Aabb*, 1/4 *aaBb*, and 1/4 *aabb*. If the loci are linked, the frequency of the four genotypes will deviate significantly from the 1:1:1:1 ratio.

AaBb* × *aabb

Independent assortment		Coupling linkage		Repulsion linkage	
♀	♂ *ab*	♀	♂ *ab*	♀	♂ *ab*
$\frac{1}{4}$ *AB*	$\frac{1}{4}$ *AaBb*	*>$\frac{1}{4}$ *AB*	>$\frac{1}{4}$ *AaBb*	<$\frac{1}{4}$ *AB*	<$\frac{1}{4}$ *AaBb*
$\frac{1}{4}$ *Ab*	$\frac{1}{4}$ *Aabb*	<$\frac{1}{4}$ *Ab*	<$\frac{1}{4}$ *Aabb*	>$\frac{1}{4}$ *Ab*	>$\frac{1}{4}$ *Aabb*
$\frac{1}{4}$ *aB*	$\frac{1}{4}$ *aaBb*	<$\frac{1}{4}$ *aB*	<$\frac{1}{4}$ *aaBb*	>$\frac{1}{4}$ *aB*	>$\frac{1}{4}$ *aaBb*
$\frac{1}{4}$ *ab*	$\frac{1}{4}$ *aabb*	>$\frac{1}{4}$ *ab*	>$\frac{1}{4}$ *aabb*	<$\frac{1}{4}$ *ab*	<$\frac{1}{4}$ *aabb*

*>, greater than; <, less than.

A second procedure for determining the degree of linkage is to self-pollinate individuals that are heterozygous at two loci. If there is complete dominance at each locus and no epistasis, the average ratio of the progeny will be 9 *A__B__*: 3 *A__bb*: 3 *aaB__*: 1 *aabb*. Linkage in coupling or repulsion will cause significant deviations from the 9:3:3:1 ratio.

Progeny from self-pollination of an *AaBb* individual

Linkage Type	Gametic Ratio	Phenotypic Ratio with Complete Dominance
Independent	$\frac{1}{4}$ *AB*:$\frac{1}{4}$ *Ab*:$\frac{1}{4}$ *aB*:$\frac{1}{4}$ *ab*	9 *A__B__*:3 *A_bb*:3 *aaB__*: 1 *aabb*
Coupling*	>$\frac{1}{4}$ *AB*:<$\frac{1}{4}$ *Ab*:<$\frac{1}{4}$ *aB*:>$\frac{1}{4}$ *ab*	>9 *A__B__*:<3 *A__bb*:<3 *aaBb*:>1 *aabb*
Repulsion	<$\frac{1}{4}$ *AB*:>$\frac{1}{4}$ *Ab*:>$\frac{1}{4}$ *aB*:<$\frac{1}{4}$ *ab*	<9 *A__B__*:>3 *A__bb*:>3 *aaBb*:<1 *aabb*

Pattern of Segregation with Coupling Linkage

	>$\frac{1}{4}$ *AB*	<$\frac{1}{4}$ *Ab*	<$\frac{1}{4}$ *aB*	>$\frac{1}{4}$ *ab*
>$\frac{1}{4}$ *AB*	>AABB	<*AABb*	<*AaBb*	>*AaBb*
<$\frac{1}{4}$ *Ab*	<*AABb*	<*AAbb*	<*AaBb*	<*Aabb*
<$\frac{1}{4}$ *aB*	<*AaBB*	<*AaBb*	<*aaBB*	<*aaBb*
>$\frac{1}{4}$ *ab*	>*AaBb*	<*Aabb*	<*aaBb*	>*aabb*

*>, greater than; <, less than.

Table 3-4 Progeny from the Cross *PpTt* × *pptt* in Poppy

	Genotype				
Frequencies	*PpTt*	*Pptt*	*ppTt*	*pptt*	Total
Observed	191	37	36	203	467
Expected (with no linkage)	116.75	116.75	116.75	116.75	467
Class description	Parental	Nonparental	Nonparental	Parental	

Source: Philip, 1934, as discussed by Mather, 1951.

Two loci are considered independent if the recombination percentage between them is 50 percent. The recombination percentage also is commonly referred to as the number of map units between genes when the value is significantly less than 50 percent. There are several methods for calculating recombination percentage. Examples will be given for two methods to illustrate how the distance between genes on a chromosome is determined.

Mather (1951) illustrated one method of calculation using the data of Philip (1934) in the poppy (Table 3-4). A heterozygous individual was crossed to a homozygous recessive, *PpTt* × *pptt*. The observed genotypic frequencies did not reflect independent assortment because the number of *PpTt* and *pptt* individuals was greater than and the number of *Pptt* and *ppTt* individuals smaller than expected. The loci were considered linked in coupling because the *PpTt* and *pptt* classes were in highest frequency. The percentage recombination, also expressed as the number of map units between loci, was calculated from the equation

$$\text{Recombination } \% = \frac{\text{sum of nonparental classes}}{\text{total number of individuals}} \times 100$$

$$= \frac{Pptt + pptt}{PpTt + Pptt + ppTt + pptt} \times 100$$

$$15.63\% = \frac{37 + 36}{191 + 37 + 36 + 203} \times 100$$

The product-moment method was developed by Immer (1930) to determine the percentage of recombination when F_2 individuals are obtained from self-pollination of a heterozygous individual. For loci in repulsion, the formula is

$$\frac{(\text{Number of } A\_\_B\_\_)\ (\text{number of } aabb)}{(\text{Number of } A\_\_bb)\ (\text{number of } aaB\_\_)}$$

For coupling, the formula is inverted to become

$$\frac{(\text{Number of } A\_\_bb)\ (\text{number of } aaB\_\_)}{(\text{Number of } A\_\_B\_\_)\ (\text{number of } aabb)}$$

Immer (1930) developed a table with which the ratio of products can be converted to the percentage of recombination.

An example of the product-moment method was provided by Immer and Henderson (1943). They evaluated the linkage between the locus for non-six-rowed (V) versus six-rowed (v) heads of barley and the locus for awnless (E) versus awned (e) outer glumes (Table 3-5). The selfed progeny of a heterozygote had a greater frequency of $V\_\_E\_\_$ and $vvee$ genotypes than expected with independent assortment, indicating that the loci were linked in the coupling phase. The ratio of the products was

$$\frac{(219\ V\_\_ee)\ (246\ vvE\_\_)}{(1221\ V\_\_E\_\_)\ (243\ vvee)} = 0.1816$$

The ratio of 0.1816 was equivalent to a recombination percentage of 28 percent.

Estimates of recombination percentage are subject to sampling error. Details on estimating the amount of sampling error and on other experimental and statistical procedures for linkage determination are provided by Immer (1930), Kramer and Burnham (1947), Mather (1951), and Allard (1956).

Influence of Linkage on Selection. Linkage between two or more loci that control different characters can be an advantage when desirable alleles at each locus are in coupling. The advantage is that the desired alleles will occur together more frequently in a segregating population than would be expected with independent assortment, so the breeder has less difficulty obtaining individuals with the desirable alleles for two characters. Linkage is undesirable when desirable alleles are in repulsion. The breeder must grow larger populations than with unlinked loci to obtain segregates with the desired alleles on the same chromosome as a result of a crossover between two loci linked in repulsion.

Wiebe (1960) presented an example of how linkage could be used by the breeder. He proposed that hybrid barley could be produced with genetic male sterility if the gene controlling male sterility was tightly linked to a gene controlling sensitivity to a chemical, such as DDT. The proposal has never become a reality because a close linkage between male sterility and chemical sensitivity has never been found.

Table 3-5 F_2 Progeny from Self-Pollination of Heterozygous Individuals in Coupling Phase for Two Characters of Barley, *VE/ve*

	Genotype				
Frequencies	$V\_\_E\_\_$	$V\_\_ee$	$vvE\_\_$	$vvee$	Total
Observed	1221	219	246	243	1929
Expected (with no linkage)	1085.0	361.7	361.7	120.6	1929

Source: Immer and Henderson, 1943.

An unfavorable linkage was reported by Matson and Williams (1965) in breeding for resistance to the soybean cyst nematode. An old cultivar, Peking, had the desired resistance to the disease but its seed coat was colored, an undesirable characteristic for current soybean cultivars. The authors attempted to transfer the genes for resistance from Peking to a cultivar with yellow seed coat, but all true-breeding resistant lines had a colored seed coat. Resistant yellow-seeded plants segregated for both seed coat color and nematode resistance, indicating they were heterozygous for both characters. Matson and Williams assumed that the heterozygous plants had a dominant allele (Rhg_4) for resistance linked to a recessive allele (i^i) for seed coat color, and a recessive allele (rhg_4) for susceptibility linked to the dominant allele (I) for yellow seed coat. To break the repulsion linkage, they grew the progeny from one yellow-seeded resistant F_2 plant and selected and progeny-tested yellow-seeded resistant F_3 segregates. They assumed that the genotype of the F_2 plant was Rhg_4 i^i/rhg$_4$ I and that an effective crossover would produce F_3 segregates of the genotype Rhg_4I/rhg_4I. The progeny of the desired F_3 would segregate for resistance, but be homogeneous for yellow seed coat. No crossover individuals were found in F_3; therefore, selfed progeny of yellow-seeded F_3 plants were grown and evaluated as in the previous generation, but again the desired crossover was not found. The process was repeated until one homozygous, yellow-seeded, resistant progeny was found in F_7 and another in F_8. Matson and Williams determined that the locus for resistance and the one for seed coat had a recombination percentage of only 0.35 percent.

Linkage Versus Pleiotropy. A tight linkage between two loci can be confused with pleiotropy, the control of two or more characters by a single gene. For example, the linkage of resistance to the soybean cyst nematode and seed coat color seemed to be a case of pleiotropy because the two characters were always inherited together (Matson and Williams, 1965). The only way linkage and pleiotropy could be distinguished was by finding a crossover product, such as a progeny homozygous for resistance and yellow seed coat. Resistance and yellow seed coat could never occur in a true-breeding individual if pleiotropy was present. Thousands of individuals may have to be grown to break a tight linkage that seems to be one of pleiotropy.

Effect of Linkage on Genetic Variability. Linkage reduces the frequency of certain genotypes and increases the frequency of others. A population is in linkage disequilibrium when the frequency of gametes with genes in coupling is not equal to the frequency of gametes with genes in repulsion ($AB + ab \neq Ab + aB$). Linkage disequilibrium is most common in populations developed from two inbred parents with contrasting strengths and weaknesses. For achievement of linkage equilibrium in a population, the opportunity must be provided for genetic recombination within heterozygous individuals. This requires repeated generations of intermating or selfing of heterozygous individuals.

Heterozygosity is essential for the breakup of linked genes. An effective crossover is one that results in a new combination of linked genes, whereas an ineffective crossover does not produce any new genetic types. Effective crossovers can only occur when both of the linked loci are heterozygous, *Ab/aB* or *AB/ab*. A crossover in an *Ab/aB* individual results in the new products *AB* and *ab*, and in an *AB/ab* individual produces *Ab* and *aB* gametes. A crossover in an individual that is homozygous at one or both loci is ineffective. *Ab/AB* individuals can only produce *Ab* and *AB* gametes and *AB/AB* genotypes can only produce *AB* gametes, regardless of the amount of crossing over.

Groups of genes that are linked and tend to be transmitted intact from one generation to the next are referred to as linkage blocks. Breeders often transfer major genes from one cultivar to another by backcrossing. They are aware, however, that they are not transferring only one locus, but rather a block of loci that are closely linked to the one of interest. For that reason, the original cultivar and the new version developed by backcrossing are not truly isogenic lines. Isogenic lines are genetically identical except for one locus. Instead, some persons prefer to call them near-isogenic lines, which suggests that they differ by a block of genes linked to the gene of interest.

The length of linkage blocks that are retained in a breeding population is influenced by the number of parents used to develop the population, the number of generations of intermating (hybridization) before selfing is initiated, and the number of selfing generations conducted after intermating is completed. A series of theoretical studies were reported by Hanson (1959a,b,c) with respect to the lengths of linkage blocks present in homozygous parents that remain intact after generations of intermating and self-pollination.

Linkage can influence estimates of genetic variance for quantitative characters. An example of the impact of linkage on the estimation of types of gene action and the partitioning of genetic variance into additive and dominance components was provided by Gardner and Lonnquist (1959). They provided evidence that repulsion phase linkages in a segregating population gave upward biased estimates of overdominance and downward biased estimates of additive genetic variance for genes controlling yield.

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CHAPTER FOUR

Polyploidy

The chromosome makeup of a crop species can influence the breeding procedures that are used for cultivar development. The number and origin of the chromosomes influence such factors as the amount of loss in vigor occurring with self-pollination, the type of hybrid that will exhibit the maximum heterosis, the most practical strategy for backcrossing, and the feasibility of obtaining useful characteristics from other species.

A genome is the basic unit used to describe the chromosome makeup of an individual. With normal meiosis and mitosis, the chromosomes of a genome are inherited together as a set (Chap. 2). Different genomes in a group of related species commonly are assigned capital letters (e.g., A, B, C), and the number of chromosomes in each genome is designated by x.

A euploid contains a multiple of the basic chromosome number. A monoploid has one set of chromosomes (x) and a diploid has two (2x). Individuals with more than two sets of chromosomes are referred to as polyploids, which can be triploid (3x), tetraploid (4x), pentaploid (5x), hexaploid (6x), and so forth. A polyploid that has more than two sets of a single genome is an autoploid, and a polyploid that possesses two or more different genomes is an alloploid. Consider two genomes designated A and B. An autotetraploid (4x) would have four multiples of either the A or the B genome. An allotetraploid (4x) would have two sets of the A and two sets of the B genomes.

The number of chromosomes in a somatic cell is commonly designated 2n. The letter "n" does not indicate the number of different sets (x) of chromosomes present; therefore, it is helpful to use both x and n when describing the chromosome numbers of a genotype. The somatic cells of a diploid are $2n = 2x$ and the gametes are $n = x$. For an autohexaploid, the somatic cells are $2n = 6x$ and the gametes are $n = 3x$.

The number of sets of chromosomes in a haploid (n) individual or gamete is half the number present in the somatic cells of the individual from which the

gamete was derived. In an autoploid crop, there may be individuals with the same genome at different ploidy levels: diploid, tetraploid, hexaploid, and so forth. To relate the chromosome number of haploids derived from individuals of different ploidy levels for a single genome, a prefix is added to the word "haploid" to denote the number of sets (x) of the basic genome present. A monohaploid (n = 1x) is derived from a diploid, a dihaploid (n = 2x) from a tetraploid, and a trihaploid (3x = n) from a hexaploid.

GENETICS OF AUTOPLOIDS

An autoploid may have more complex segregation ratios for alleles at a single locus than those obtained with diploid individuals. The number of different alleles that an individual can possess for a single locus on a chromosome is equal to the individual's ploidy level. An autotetraploid can have up to four different alleles and an autohexaploid up to six different alleles. The multiallelism of an autoploid influences its response to self-pollination, sib mating, backcrossing, and other breeding procedures. The response can be understood by considering the segregation of alleles at a single locus in an autotetraploid.

Five different genotypes are possible at a locus of an autotetraploid. The genotypes can be defined on the basis of the number of times a dominant (*A*) or a recessive (*a*) allele is present. There are no dominant alleles in a nulliplex genotype, one in a simplex, two in a duplex, three in a triplex, and four in a quadriplex.

Term	Genotype
Nulliplex	*aaaa*
Simplex	*Aaaa*
Duplex	*AAaa*
Triplex	*AAAa*
Quadriplex	*AAAA*

Another way to define the five genotypes is by considering four different alleles at a locus (Busbice and Wilsie, 1966). The four alleles are designated *a*, *b*, *c*, and *d* to avoid any connotation of dominance.

Only four nulliplex genotypes are possible (*aaaa*, *bbbb*, *cccc*, and *dddd*) and only one tetragenic genotype (*abcd*). Many different combinations of alleles are possible for the simplex, duplex, and trigenic genotypes.

Intraallelic and interallelic interactions may occur for as many as four alleles per locus in a tetraploid. Intraallelic interaction between two different alleles is referred to as a first-order interaction, among three alleles is a second-order interaction, and among four alleles is a third-order interaction.

The gametes obtained from a tetraploid are dihaploid (2x). The number of

Term	Genotype Description	Examples
Nulliplex	Only one allele present	*aaaa*, *bbbb*
Simplex	One allele present in three copies, another allele present in one copy	*aaab*, *abbb*
Duplex	Two alleles, each present in two copies	*aabb*, *bbcc*
Trigenic	One allele present in two copies, two different alleles present in one copy	*aabc*, *bbcd*
Tetragenic	Four different alleles present	*abcd*

different gametes produced by each tetraploid genotype ranges from one for a nulliplex to six for a tetragenic individual.

Genotype	Gametic Array
Nulliplex, *aaaa*	aa
Simplex, *aaab*	$aa + ab$
Duplex, *aabb*	$aa + 4ab + bb$
Trigenic, *aabc*	$aa + 2ab + 2ac + bc$
Tetragenic, *abcd*	$ab + ac + ad + bc + bd + cd$

The genotypic array in S_1 obtained by the self-pollination of an S_0 plant is equal to the square of the gametic array:

S_0 Plant	Genotypic Array of S_1 Progeny
$aaaa \rightarrow$	$(aa)^2$ = all nulliplex
$aaab \rightarrow$	$(aa + ab)^2 = \frac{1}{4}$ duplex $+ \frac{1}{2}$ simplex $+ \frac{1}{4}$ nulliplex
$aabb \rightarrow$	$(aa + 4ab + bb)^2 = \frac{1}{2}$ duplex $+ \frac{4}{9}$ simplex $+ \frac{1}{18}$ nulliplex
$aabc \rightarrow$	$(aa + 2ab + 2ac + bc) = \frac{1}{2}$ trigenic $+ \frac{1}{4}$ duplex $+ \frac{2}{9}$ trigenic $+ \frac{1}{6}$ duplex
$abcd \rightarrow$	$(ab + ac + ad + bc + bd + cd)^2 = \frac{1}{6}$ tetragenic $+ \frac{2}{3}$ trigenic $+ \frac{1}{6}$ duplex

The expression of heterosis and inbreeding depression in an autotetraploid has been attributed to the degree to which intraallelic interaction can occur. The influence of this concept on breeding procedures is discussed in Chap. 8 and 9.

GENETICS OF ALLOPLOIDS

The inheritance patterns of alloploids are usually less complex than those of autoploids and in some cases follow true diploid genetics. There may, however, be multiallelism among the different genomes of an alloploid. This multiallelism will cause inheritance patterns for genes at these loci to be more complex than those of diploids.

NATURAL POLYPLOIDY

Polyploidy can be produced in nature by two processes: (a) Somatic doubling may occur due to irregularities during mitosis in the meristematic cells. (b) Chromosome sets may not separate due to irregular reductional division during meiosis, and therefore, the gametes have twice the expected chromosome number. It has been estimated that approximately 70 percent of grass species and 23 percent of legume species are polyploid. There are relatively few crop species in which natural autoploidy is known to occur, and these include potato, alfalfa, coffee, peanut, and banana. Autotetraploid species of potato possess a mechanism that promotes bivalent formation during meiosis, which allows plants to maintain a high degree of fertility (Plaisted, 1980).

Alloploidy occurs more commonly in crop species than does autoploidy. Oat, wheat, cotton, tobacco, and sugarcane are examples of crops having one or more natural alloploid species. Many of these crops or their wild relatives also have diploid species. Natural selection has led to a high degree of fertility in natural alloploids. The evolutionary success of alloploids had been attributed to their vegetative vigor, hybridity, and genetic and evolutionary flexibility (Simmonds, 1979).

INDUCED POLYPLOIDY

The somatic chromosome number of a plant can be doubled by a number of physical and chemical agents. Colchicine, a natural alkaloid extracted from the autumn crocus, has been the chemical most commonly utilized. Colchicum and calcemid are synthetically derived forms of colchicine. The chemical is *applied to the meristematic region of a plant, where cells are undergoing mitosis. The chromosomes in the cells duplicate normally, but spindle fibers do* not form. The chromosomes fail to line up at the equatorial plate during metaphase, and normal anaphase and telophase do not occur. The result is a polyploid cell with twice the original chromosome number.

The role of induced polyploidy in plant breeding has been reviewed by Dewey (1980). The three uses of induced polyploidy have been to produce autoploids by increasing the chromosome number of an existing species, to produce am-

phiploids by increasing the chromosome number of a hybrid produced from the cross of two species with different genomes, and to facilitate gene transfer between genotypes with different ploidy levels or between species. The first two uses of induced polyploidy will be reviewed in this chapter. The third use is discussed in Chap. 14.

Four factors should be considered in the use of induced autoploidy or alloploidy for plant improvement (Dewey, 1980).

1. *Ploidy level:* Existing crop species probably have an optimum ploidy level, and any increase is likely to be more detrimental than beneficial. Research with different levels of induced polyploidy indicates that increasing the chromosome number beyond the hexaploid level is of little or no advantage. Doubling the chromosome number of a tetraploid or hexaploid would not be recommended if the goal were to develop a genotype that had the potential to increase agricultural production.
2. *Plant part of economic value:* Induced polyploidy can cause irregularities of chromosome pairing, which result in reduced frequencies of viable gametes and seed set. Induced polyploidy would have the greatest chance of success for a species that is vegetatively propagated and whose vegetative parts are of economic value. A species valued for its vegetative parts but propagated by seed would offer the second greatest chance of success. The most difficult use of induced polyploidy would be in a species grown commercially for its seed.
3. *Economic importance of the crop:* A crop with a new ploidy level is likely to have weaknesses that must be overcome before it is commercially acceptable. Multiple generations of hybridization and selection generally are necessary before the breeding potential of the induced polyploid can be determined. A large investment of time and money is required to determine if a new ploidy level will make the crop superior to existing species. Such an investment can be most readily justified for a crop that has potential for large economic returns.
4. *Length of a selection cycle:* The use of induced polyploidy, as just noted, generally requires multiple generations of hybridization and selection. The length of time required to complete a cycle of selection determines the number of years before useful cultivars can be obtained. Induced polyploidy is more attractive for annual crops with relatively short selection cycles than for perennial species that must be grown for several years before they can be evaluated.

Induced Autoploidy

Two common effects of doubling the chromosome number of an existing species are an increase in the size of plant parts and a decrease in seed production. The increase in size of plant parts is due to larger cell size. The decrease in seed

production is associated with reduced frequency of viable gametes caused by the irregularity of chromosome paring.

The use of induced autoploids in crop production has been limited, despite the fact that they have been studied in every major crop and in most minor crops. Decreased seed production has been the primary reason for their failure. The species that should benefit most from the increase in plant size are those grown for their vegetative parts, such as the roots of sugarbeet or the forage of red clover. Even in these species, failure to obtain adequate seed quantities at a reasonable price for commercial plantings has been a major obstacle. For example, autotetraploid red clover is superior in forage yield to the diploid in some countries, but is used only to a minor extent due to the higher cost of the tetraploid seed. Autotetraploid grapes have larger fruit size than do diploids but are not grown because of erratic fruit set. Autotriploidy has been utilized in some species, such as watermelon, to obtain seedless fruit. However, triploid production is costly and feasible only for crops in which individual fruits have a high economic value.

Induced Alloploidy

Induced alloploids are formed by mating two species with different genomes and doubling the chromosome number of the hybrid. Such a polyploid that contains the full chromosome complement of two species is also referred to as an amphiploid.

Amphiploids are a potential source of new crop species. Despite this potential, the only example of a new species grown commercially is triticale (X *Triticosecale*), an amphiploid from the cross between wheat (*Triticum*) and rye (*Secale*). Four reasons were suggested by Dewey (1980) for the lack of success in developing new species: (a) inappropriate choice of parental species, (b) inadequate genetic variation in the initial breeding populations, (c) inadequate number of cycles of selection before programs were abandoned, and (d) failure to work with low ploidy levels.

The importance of choosing the appropriate parental species is related to the problem of incomplete seed set in amphiploids. The chromosomes from different species often exhibit some degree of pairing in an amphiploid, and inviable gametes are produced that have more or fewer chromosomes than the complete haploid complement. Incomplete seed set is less of a problem in a crop produced for its vegetative parts than in one produced for seed.

Genetic variation in the initial breeding populations is determined by the number of genotypes of two species that are mated. It often is difficult to obtain F_1 seed from the interspecific matings used to produce amphiploids. When relatively few genotypes of two species are used, the genetic variation in the initial breeding populations will be limited. The lack of genetic variability limits the success that can be expected from selection for important characteristics in the new species.

Development of commercially acceptable cultivars of a new species requires a long-term breeding program. In the development of triticale, progeny from the initial wheat × rye crosses were undesirable because of low fertility, shriveled seeds, and weak straw. Several cycles of hybridization and selection were required to develop the cultivars currently in use. There is little likelihood of success with amphiploids if breeding is discontinued when success is not immediate.

The mating of species with low ploidy levels is considered a major factor in the success of induced alloploidy. The recovery of adequate fertility has been better in tetraploid and hexaploid amphiploids than in octaploids or higher ploidy level species (Dewey, 1980).

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CHAPTER FIVE

Variation in Chromosome Number and Structure

Plants whose chromosomes deviate in number and structure from the basic genomes found in normal individuals have been found in many species. The deviants have been important tools in genetic studies, and some of them have been incorporated into schemes for cultivar development. A brief overview of the most common types of aberrations and their proposed applications to plant breeding is provided in this chapter. A detailed description of the cytogenetics of aneuploids is provided by Khush (1973).

ANEUPLOIDY

Types of Aneuploidy

The chromosome number in normal somatic cells of a plant species is a multiple of some basic monoploid (x) number. Diploid individuals having two homologous pairs of each chromosome (2x) are referred to as disomic. A variation in chromosome number that does not involve multiples of the monoploid number is referred to as aneuploidy. The variation may involve the addition or deletion of whole chromosomes or parts of chromosomes (Table 5-1).

The concept of aneuploidy will be illustrated here with three chromosomes of a diploid species. The chromosomes are designated AA, BB, and CC. Arms of chromosomes are given dashed numbers, -1 and -2. A-1 and A-2 are the two arms of chromosome A. The number of chromosomes of A, B, and C is symbolized by roman numerals: one (univalent) as I, a pair (bivalent) as II, three (trivalent) as III, four (quadrivalent) as IV, and so forth. For example, 3II signifies three pairs of chromosomes in a normal diploid individual. A genotype designated II + I + III has one pair of chromosomes plus a univalent plus a trivalent.

Table 5-1 Common Types of Aneuploidy Include Addition or Deletion of Entire Chromosomes or Parts of Chromosomes from the Basic Somatic Number (2n) in a Species

Type of Aneuploidy	Chromosome Number*
Disomic	2n
Chromosome additions:	
Primary trisomic	2n + 1A
Double trisomic	2n + 1A + 1B
Tetrasomic	2n + 2A
Secondary trisomic	2n + isochromosome A
Telosomic trisomic	2n + telocentric A
Tertiary trisomic	2n + interchange A
Chromosome deletions:	
Monosomic	2n − 1A
Nullisomic	2n − 2A
Monoisodisomic	2n − 2A + isochromosome A
Monotelosomic	2n − 2A + telocentric A

*A and B are different chromosomes of the same genome. The number preceding the letter designates the number of copies of the chromosome that are involved in the aneuploid.

Chromosome Additions. Several types of aneuploids are formed by the addition of entire chromosomes or parts of chromosomes to the disomic (2n) complement.

1. Primary trisomics have one additional chromosome in their somatic cells, 2n + 1. The number of different primary trisomics that are possible in a species is equal to the number of different chromosomes in its genomes. The primary trisomics that are possible with three chromosomes (2II + III) include AAABBCC, AABBBCC, and AABBCCC.
2. Double trisomics have an extra chromosome for each of two different chromosomes in a genome, 2n + 1 + 1. A double trisomic can be formed for each pairwise combination of different chromosomes in a species. For three chromosomes, the double trisomics (II + 2III) include AAABBBCC, AAABBCCC, and AABBBCCC.
3. Tetrasomics have two extra chromosomes for one member of a genome, 2n + 2. There can be a different tetrasomic (2II + IV) for every chromosome in a genome: AAAABBCC, AABBBBCC and AABBCCCC.
4. Polysomics that have more than two extra chromosomes for one member of a genome are designated pentasomic (2n + 3), hexasomic (2n + 4), and so forth. The prefix indicates the total number of times one member of a genome is present.
5. Secondary trisomics have an additional isochromosome, 2n + isochromosome. An isochromosome has two identical chromosome arms instead of two different arms. The number of possible secondary trisomics in a species is equal to the number of different chromosome arms in the

genomes. With three different chromosomes in a genome and two arms on each chromosome, the number of possible secondary trisomics is six. As an illustration of a secondary trisomic, assume that one arm of chromosome C, designated C-1, is involved in an isochromosome C-1 • C-1. The chromosome makeup of a secondary trisomic with that isochromosome would be AABBCC(C-1 • C-1).

6. Telosomic trisomics possess an extra telocentric chromosome, 2n + telocentric. A telocentric chromosome has a chromosome arm with the centromere on one end. There can be a telosomic trisomic for each chromosome arm in a genome, or a total of six for three chromosomes in a genome. If the telocentric is one arm of chromosome C, designated C-1, the chromosome constitution of the telosomic trisomic would be AABBCC(C-1).
7. Tertiary trisomics have an extra chromosome that contains parts of two different chromosomes, 2n + interchange chromosome. An interchange chromosome is formed by a reciprocal translocation (Fig. 5-1). There are a large number of possible tertiary trisomics that differ by the pair of chromosomes involved and the amount of each chromosome in a pair that is exchanged.

Chromosome Deletions. Aneuploids can have one or more chromosomes or parts of chromosomes that are missing.

1. Monosomics lack an entire chromosome for one member of a genome, 2n − 1. The number of possible monosomics is equal to the number of different chromosomes in a genome. The monosomics (2II + I) for three chromosomes in a genome are A̲BBCC, AA̲BCC, and AABB̲C.
2. Nullisomics lack both chromosomes of a pair in a diploid species, 2n − 2. There can be a different nullisomic for each chromosome in a genome. The nullisomics (2II + 0) for three chromosomes in a diploid are AABB, AACC, and BBCC.
3. Monoisodisomics have an isochromosome in place of one pair of chromosomes; this isochromosome substitutes entirely for one arm of the chromosome pair that is lacking. The arm of the chromosome not included in the isochromosome is lacking from the nucleus. As an example of a monoisodisomic (2II + isochromosome), consider chromosome C, whose arms are designated C-1 and C-2. If the isochromosome were C-1 • C-1, the monoisodisomic would be AABB(C-1 • C-1). Chromosome arm C-2 would be lacking in the individual.
4. Monotelosomics have a telocentric chromosome instead of one pair of chromosomes. The telocentric has one arm of the missing chromosome pair; therefore, the genotype lacks a copy of one chromosome arm and lacks two copies of the other arm. If the monotelocentric (2II + 0 + telocentric) involved chromosome C and the telocentric chromosome in-

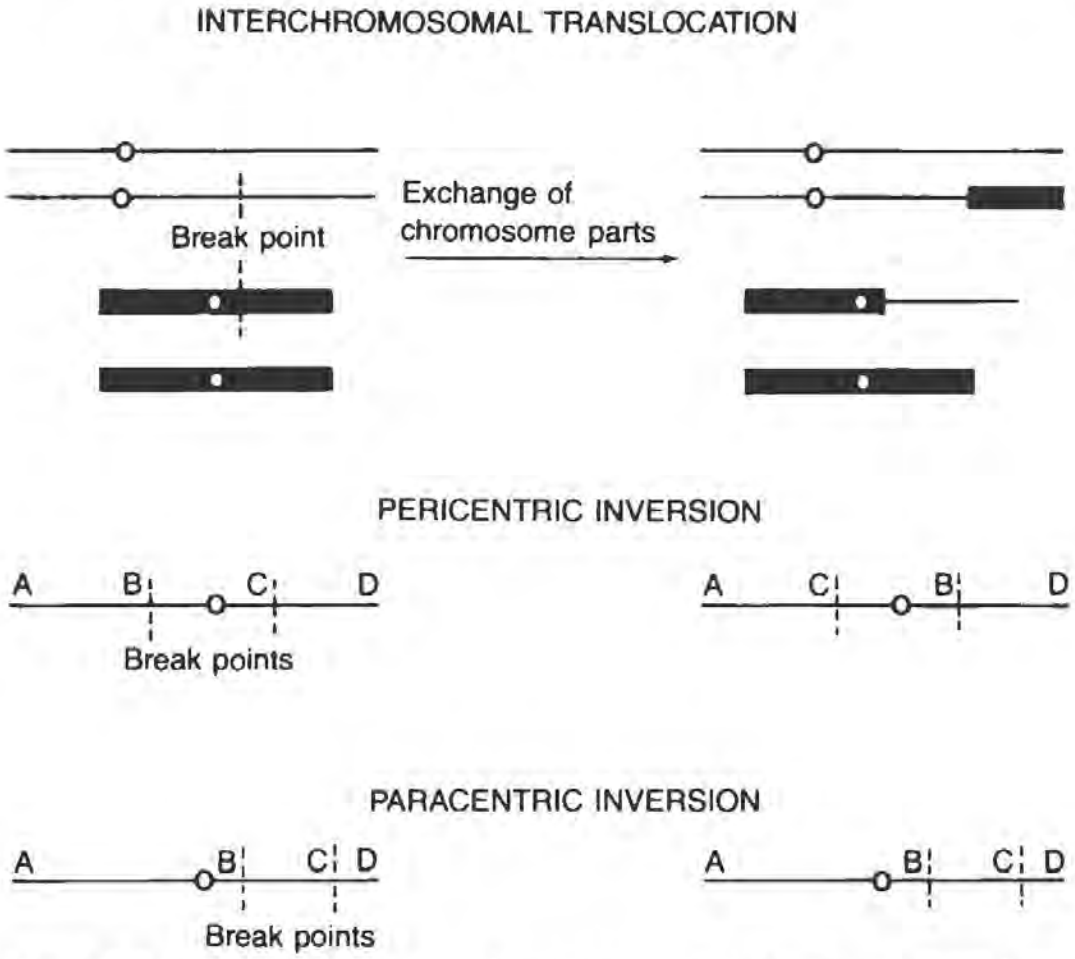


Figure 5-1 Formation of an interchromosomal translocation, a pericentric inversion, and a paracentric inversion.

cluded arm C-1, the genotype would be AABBC-1. Both copies of chromosome arm C-2 and one copy of C-1 would be lacking.

Reproduction of Aneuploids

Gamete and zygote production and survival can be markedly different for an aneuploid than for a 2n genotype. Chromosome pairing and division in an aneuploid are disrupted by the addition or deletion of part or all of a chromosome (Burnham, 1962). In a primary trisomic, for example, the three chromosomes can pair to form a trivalent, or two of the chromosomes can pair while the third remains separate as a univalent. The univalent may migrate to one of the poles to form n + 1 gametes or may lag behind and be lost.

Chromosome additions or deletions are more readily transmitted through egg cells than through pollen. The viability and vigor of pollen with chromosome additions or deletions are lower than those of pollen with the entire chromosome

complement. Transmission of some additions or deletions through the pollen does not occur, and in other cases the frequency of transmission is low. The abnormal pollen may not germinate, or slow pollen tube growth may prevent the sperm nucleus from reaching the embryo sac as rapidly as in normal pollen. If fertilization occurs, an aneuploid zygote is more likely to abort than is a diploid zygote. The size and vigor of aneuploid seeds and plants that develop from them also may be reduced.

Applications

The reproductive behavior of cytologically aberrant plants has been exploited in developing systems to facilitate hybrid seed production and for research on plant genetics.

Hybrid Seed Production. Ramage (1965) proposed a system that would utilize a tertiary trisomic of the female parent to obtain the male-sterile plants required for hybrid seed production of a self-pollinated species such as barley. The interchange chromosome in the tertiary trisomic would possess a tight linkage between the interchange break point and a dominant allele for male fertility (*Ms*). The two normal chromosomes of the tertiary trisomic would have the recessive allele for male sterility (*ms*).

The interchange chromosome would not be transmitted through the pollen; therefore, all of the functional male gametes would have the normal chromosome with the *ms* allele. The interchange chromosome would not be transmitted through the egg cells as readily as the normal chromosomes, consequently, about 70 percent of the functional female gametes would have the normal chromosome with the *ms* allele. The majority of the other functional female gametes would have the extra interchange chromosome. As a result, about 70% of the selfed progeny of a tertiary trisomic in barley would be male-sterile diploids (*msms*), and the majority of the other 30 percent of the selfed progeny would be male-fertile tertiary trisomics (*Msmsms*).

To obtain seed of the female parent for hybrid seed production, self-pollinated seed of the tertiary trisomic would be sown. Egg cells from the male-sterile diploid plants (*msms*) would be fertilized by pollen with the *ms* allele from the tertiary trisomic plants. Therefore, all the seed harvested from the diploid plants would be homozygous for male sterility (*msms*). The seed harvested from the diploid plants would be used to grow the female parent in a hybrid seed production field. The male or pollinator parent for hybrid seed production would be a normal diploid line that was homozygous for male fertility (*MsMs*).

The Ramage system in not currently used to produce hybrid barley. One limitation of the system is the time required to incorporate the interchange chromosome into lines that would be used as a female parent.

Chromosome Mapping. Chromosome additions and deletions have been used successfully for identifying the chromosome on which genes are located and the distance between genes within a linkage group (Burnham, 1962). Primary trisomics and monosomics have been useful for locating genes on specific chromosomes. Secondary and telosomic trisomics have been used to locate genes on chromosome arms. The distance of genes from the centromere has been determined with telosomic trisomics.

The use of aneuploids for chromosome mapping will be illustrated here with monosomics. The procedure can be described by determining the location of gene *S* in a species for which transmission of n − 1 gametes occurs only through the egg. The missing chromosome is designated with a 0. The outcome of crosses depends on whether the dominant (*S*) or recessive (*s*) allele is in the monosomic.

To determine the chromosome on which a dominant allele is located, each monosomic is crossed as female to a genotype that is homozygous for the recessive allele controlling the character (Table 5–1). If the gene is not located on the missing chromosome in a monosomic, the F_1 plants will all have the dominant phenotype. If the gene is on the missing chromosome, segregation for the recessive and dominant phenotype will be observed. The recessive phenotype will be expressed in the monosomic progeny and the dominant phenotype in disomic individuals.

Table 5–1 Crosses with Monosomics to Determine the Location of a Dominant Allele

Crosses Possible	F_1
Gene not on missing chromosome, *SS* × *ss*	*Ss* dominant
Gene on missing chromosome, *S*0 × *ss*	*Ss* dominant *s*0 recessive

To establish the chromosome on which a recessive allele occurs, the monosomics in a set are crossed as female to a homozygous-dominant individual (Table 5–2). All of the F_1 plants will have the dominant genotype, whether they are monosomic or disomic. Segregation in the F_2 will determine on which chromosome the gene is located. If the gene is not on the missing chromosome, the progeny of all F_1 plants will segregate in a 3:1 phenotypic ratio. If the gene is on the missing chromosome, the monosomic F_1 plants will have no recessive phenotypes in their F_2 progeny.

Chromosome Substitution. The transfer of intact chromosomes from one cultivar to another within the same or a different species has been accomplished by the use of monosomics, nullisomics, monotelosomics, and monoisodisomics (Burnham, 1962). The techniques were developed with wheat and have been used in that species. A genotype with an extra chromosome or chromosome pair from another species is referred to as an alien addition line or race. An alien substitution

Table 5–2 Crosses with Monosomics to Determine the Location of a Recessive Allele

Generation	Crosses Possible	
	Gene Not on Missing Chromosome	Gene on Missing Chromosome
	ss × *SS*	*s0* × *SS*
F_1	*Ss* dominant	*Ss* ; *S0*
F_2	¼ *SS* ; ½ *Ss* dominant	¼ *SS* ; ½ *Ss* dominant ; *SS* ; *S0* dominant
	¼ *ss* recessive	¼ *ss* recessive

line results when a chromosome or chromosome pair donated by one species replaces a chromosome or chromosome pair in the recipient species (Rieger et al., 1976). The transfer of chromosome segments from an alien chromosome to a chromosome of the recipient species is possible through chromosomal interchange, translocation, or crossing over. For example, rust resistance was transferred from *Aegilops umbellulata* to wheat by use of an alien addition line. X-ray treatment was used to induce chromosome breakage and facilitate the transfer of the chromosome segment carrying the rust gene from *A. umbellulata* to wheat (Sears, 1956). The primary use of substitution lines has been for basic research studies on the inheritance of qualitative and quantitative characters.

Several techniques for producing substitution lines have been developed, all of which involve transfer of a chromosome by backcrossing. Backcrossing is used to recover the genotype of the recurrent parent, except for the chromosome being transferred intact. The principle of chromosome substitution within a species can be illustrated by use of a monosomic of wheat. The monosomic line, which is the recurrent parent, has 20 chromosome pairs plus the univalent, designated 20 IIR + IR. The donor parent has the normal 21 pairs of chromosomes, designated 21 IID. The monosomic line is used as the female parent because only egg cells, not pollen, are viable when a chromosome is missing. Egg cells from the female that have the normal 21 chromosome number (21 IR) result in disomic progeny (21 IR + 21 ID) and egg cells lacking a chromosome (20 IR) result in monosomic progeny (20 IR + 21 ID). The univalent chromosome in the monosomic is from the donor parent. For each backcross (BC) generation, monosomic F_1 or BC F_1 plants are used as males in backcrossing to the monosomic recurrent parent. The recurrent parent produces egg cells that are normal (n) or lacking a chromosome (n − 1) and the monosomic hybrid produces pollen that is normal. Union of an n − 1 egg with n pollen produces monosomic progeny, the univalent chromosome being from the donor parent.

Backcrossing is continued until an adequate percentage of genes from the recurrent parent has been recovered for the 20 pairs of chromosomes. After the last backcross, the monosomic hybrid plants are self-pollinated and disomic progeny are recovered. The disomic progeny are substitution lines with genes from the recurrent parent on 20 pairs of chromosomes and genes from the donor parent on the chromosome transferred intact.

Establishing Homoeologous Groups. An alloploid contains at least two different genomes. The degree to which chromosomes of different genomes are able to compensate for each other has been used in wheat to establish homoeologous groups (Burnham, 1962). Genotypes have been developed that lack a chromosome pair (nullisomic) in one genome and have an additional chromosome pair (tetrasomic) in another genome. Each chromosome of a genome in the nullisomic condition is evaluated in combination with each chromosome of another genome in the tetrasomic condition. The characteristics of the nullisomic-tetrasomic combinations are compared with those of the nullisomic and the 2n genotype. Two chromosomes are considered to be in the same homoeologous group if the nullisomic-tetrasomic combination is more similar to the phenotype of a 2n individual than to that of the nullisomic.

ALTERED CHROMOSOME STRUCTURE

Types of Structural Abnormalities

Aberrant chromosome structure may be in the form of a translocation, an inversion, a deficiency, or a duplication. The effect of aberrant chromosome structure on gamete formation varies with the type and size of the aberrancy.

Chromosome Translocation. A chromosome translocation occurs when parts of one or more chromosomes are moved to a new position on the same or a different chromosome. An intrachromosomal translocation is the movement of part of a chromosome to a different position within the same chromosome. An interchromosomal translocation, also referred to as a reciprocal translocation, involves the movement of segments between two nonhomologous chromosomes (Fig. 5-1, 5-2). The exchange of segments between nonhomologous chromosomes has been the type of translocation of primary importance for cytogenetics and plant breeding and will be the focus of this section. For the sake of brevity, the word translocation or interchange will be used, instead of interchromosomal translocation or reciprocal translocation.

Translocations have been valuable for studies of the linkage relationships among genes. They also have been used to develop a system for producing seed of hybrid maize without manual emasculation or cytoplasmic-genetic male sterility (Fig. 5–3). The usefulness of translocations is related to their influence on gene segregation and on female and male sterility.

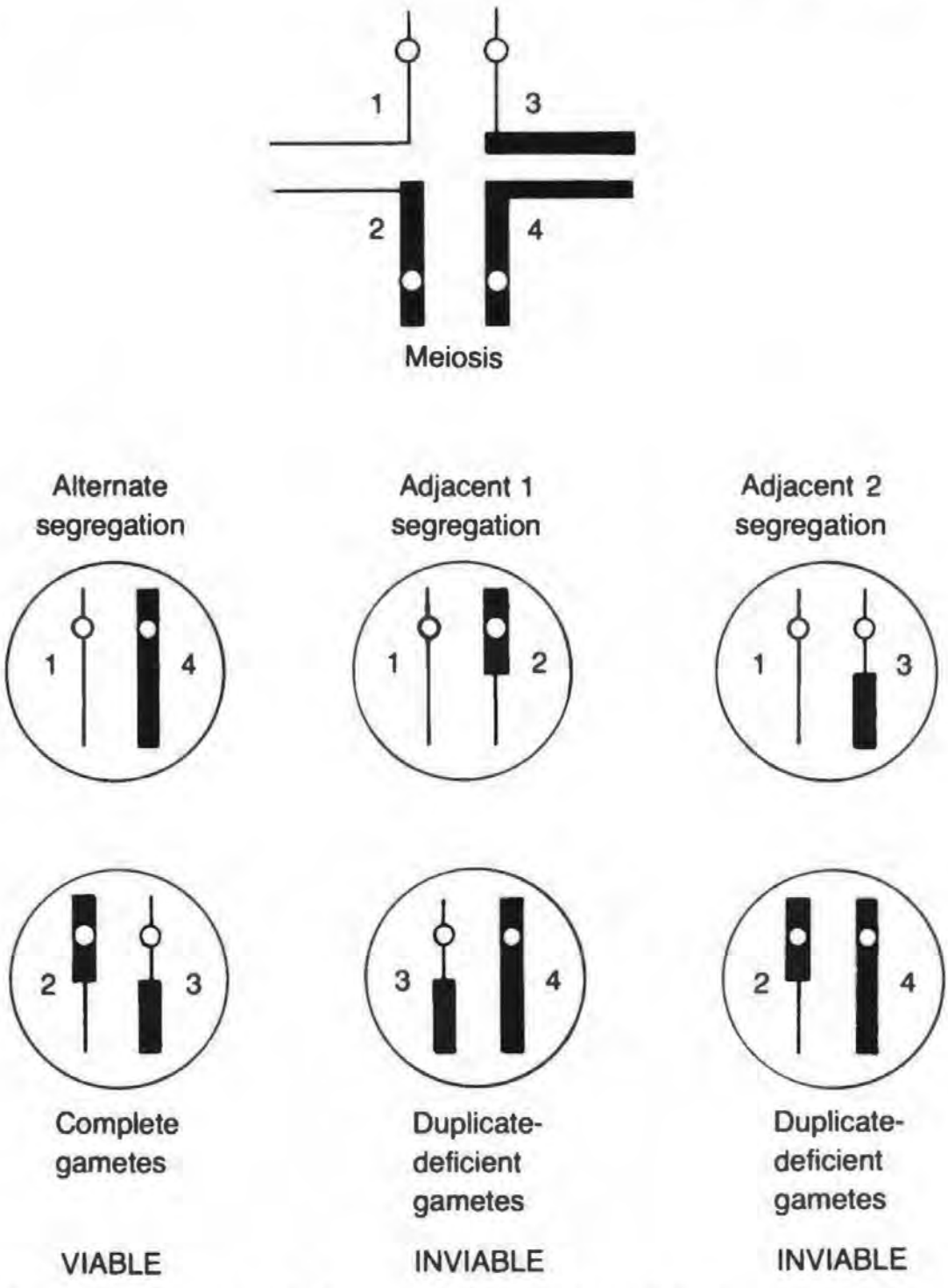


Figure 5-2 Segregation of chromosomes during meiosis in a translocation heterozygote.

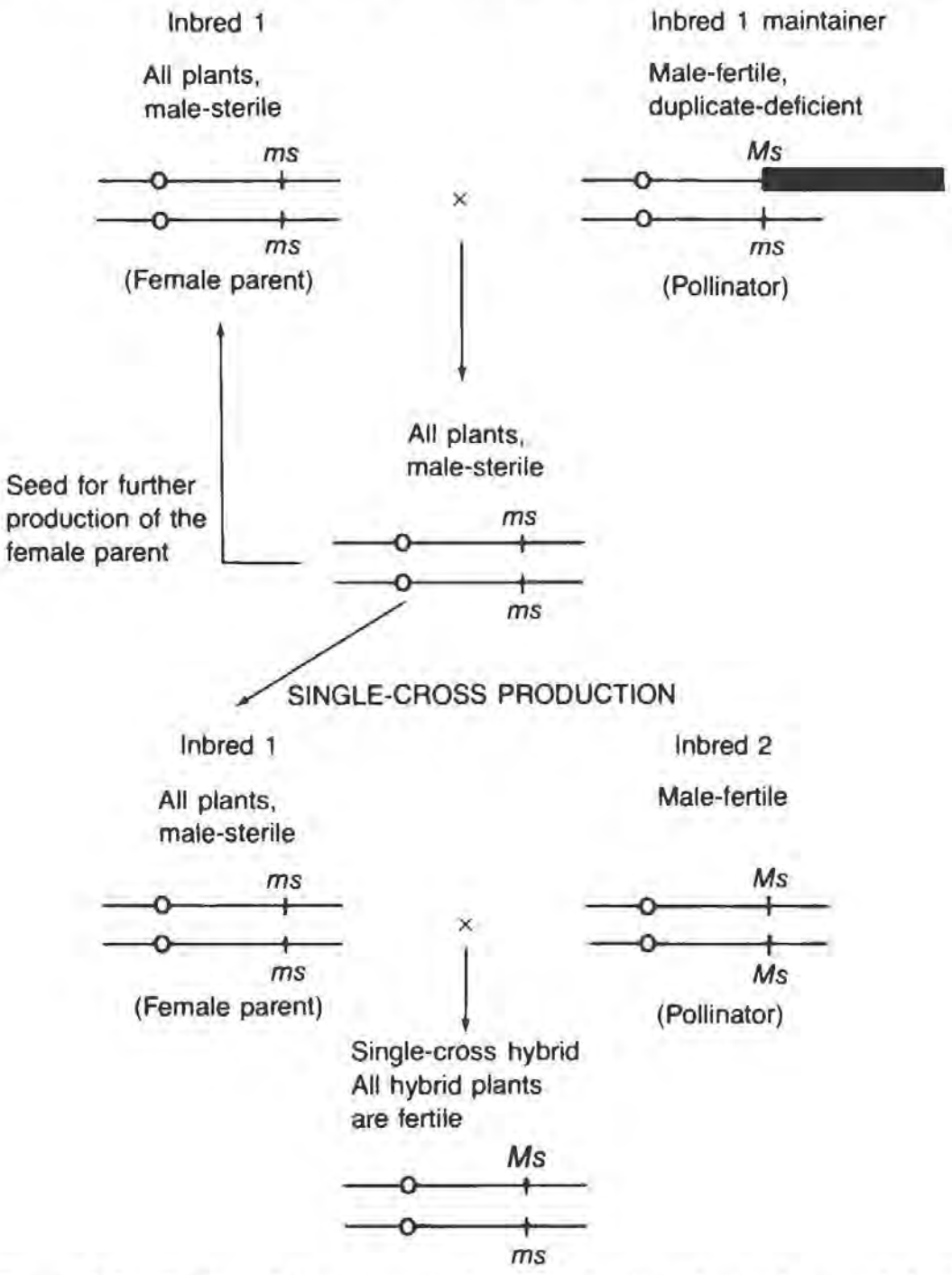


Figure 5-3 Outline of the commercial seed production of a single-cross hybrid by the use of an interchromosomal translocation and genetic male sterility, as proposed by Patterson (1975, 1978).

Chromosome Behavior. A translocation in the heterozygous condition commonly results in abortion of about half of the female and male gametes due to chromosome duplication and deficiency (Fig. 5-2). These abnormalities are associated with the nature of chromosome pairing and segregation in a heterozygous translocation. The homologous parts of chromosomes in a heterozygous translocation pair to form a cross-shaped configuration. The three kinds of segregation from the cross-configuration are alternate, adjacent 1, and adjacent 2. In alternate segregation, chromosomes with nonhomologous centromeres on opposite sides of the cross move to the same pole. The gametes produced have either two normal or two translocated chromosomes. In adjacent 1 segregation, adjacent chromosomes with nonhomologous centromeres move to the same pole. The resulting gametes have either a chromosome duplication or deficiency. Adjacent 2 segregation is movement of adjacent chromosomes with homologous centromeres to the same pole, which again results in gametes with chromosome duplication or deficiency.

The occurrence of 50 percent pollen abortion from a heterozygous translocation is related to the relative frequency of the three types of segregation and the viability of the gametes from each. About 50 percent of the gametes from a heterozygous translocation are from alternate segregation and the rest are the result of adjacent 1 or adjacent 2 segregation. The relative frequency of adjacent 1 vs. adjacent 2 segregation varies among translocations. The gametes produced by alternate segregation have no chromosome duplication or deficiency and are viable. Pollen with duplications or deficiencies from adjacent 1 or adjacent 2 segregation usually aborts. The abortion of pollen is used to identify plants that are heterozygous for a translocation. The failure of pollen to transmit chromosome duplications or deficiencies is an important factor in the proposed use of translocations for production of hybrid seed of maize.

Although pollen usually cannot tolerate a chromosome duplication or deficiency, egg cells with such abnormalities can remain functional. Transmission of abnormalities through the egg permits the formation of plants with duplications and deficiencies each generation.

Applications.

1. *Genetic studies:* Translocations have been used most extensively for cytogenetic studies, as summarized by Burnham (1962). Some of the information determined by their use includes the position of centromeres and other cytological markers in relation to genes (Anderson and Randolph, 1945), the linkage group to which genes belong, the linkage group carried by a chromosome, and the independence of linkage groups.
2. *Production of inbred lines:* Burnham (1946) proposed the use of multiple translocation stocks for inbred line production. For this purpose a multiple translocation stock is required that produces a ring of all chromosomes when crossed to normal plants. Multiple translocation stocks can be ob-

tained by crossing parents that contain different translocations. In the system proposed, an appropriate multiple translocation stock is crossed to a heterozygous stock. The only viable gametes produced by F_1 plants of this cross are those receiving either all normal (+) or all translocation (*MT*) chromosomes. Selfing the F_1 should produce *MT/MT*, *MT/+*, and +/+ progeny in a 1:2:1 ratio. The normal (+/+) progeny are homozygous for the genes of a gamete from the heterozygous stock. Consequently, a homozygous inbred line is produced in one generation.

The proposed system has not been implemented to the present time. The lack of appropriate multiple translocation stocks and the high sterility associated with heterozygosity for multiple translocations have prevented successful application of the method.

3. *Hybrid seed production:* Patterson (1975,1978) proposed the use of deficient chromosomes in facilitating production of male-sterile plants for hybrid seed production of maize. Because few simple chromosome deficiencies have been identified in maize, duplicate-deficient (*Dp-Df*) chromosomes derived from translocation heterozygotes have been utilized. A tight linkage between the translocation break point and a male-sterility locus and lack of transmission of the aberrant chromosome by the male parent are required.

   In the Patterson system, *Dp-Df* plants derived from translocation heterozygotes are crossed as male parents to male-sterile plants of the same line (Fig. 5-3). Because the *Dp-Df* chromosome is not transmitted through the male parent, the progeny of the cross are male-sterile. To be useful in crop production, crossing over between the male-sterility gene and the translocation break point, which produces transmissible *Ms* gametes, should occur at a frequency of less than 0.5 percent. Stocks that meet the required criteria are available and are being incorporated into inbred lines. Poor seed set and pollen shed in translocation stocks have limited application of this method to the present.

Chromosome Inversion. An inversion occurs when a chromosome breaks in two places and the ends of the central fragment reunite with the opposite break points of the same chromosome (Fig. 5-1) (Burnham, 1962). The two types of inversions are pericentric and paracentric. A pericentric inversion includes parts of the two chromosome arms and the centromere. A paracentric inversion involves only one arm of the chromosome. The common pairing configuration of a normal and an inverted chromosome in an inversion heterozygote is referred to as a reverse loop. The loop may not be observed during meiosis if the inversion involves a short chromosome segment.

Chromosome Behavior. Inversions can cause pollen and egg abortion due to the production of gametes with chromosome duplications and deficiencies as a result of crossing over. Inversions also are associated with reduction in the amount of

crossing over observed between genes in the inverted segment. A distinction must be made between cytological crossing over and genetic crossing over in an inversion heterozygote. Cytological crossing over refers to the exchange of segments between chromatids, regardless of its effect on genetic segregation. Genetic crossing over is measured as the percentage of recombination between genes. In an inversion heterozygote, the frequency of genetic crossing over is reduced, but cytological crossing over is not necessarily impaired. The reduction in genetic recombination is associated with the inviability of gametes produced by cytological crossing over.

Use of Inversions. There has been considerable use of inversions in cytogenetic studies (Burnham, 1962). Their uses include the study of chromosome behavior and the location of genes to particular chromosomes for qualitative and quantitative characters. No application for them in cultivar development has been found.

Chromosome Deficiency. A deficiency (deletion) is the loss of part or all of a chromosome. Deficiencies that involve part of a chromosome are formed during meiosis in translocation or inversion heterozygotes. They also may occur naturally or arise from mutagen treatment of pollen, seed, or other plant parts.

The effect of a deficiency on the viability and performance of gametes and zygotes depends on the genetic function of the fragment that is involved. A deficiency cannot be perpetuated if it causes inviability of female and male gametes. Some deficiencies are transmitted by both types of gametes and others are transmitted only through the egg.

Deficiencies have been used for gene mapping, based on the frequent expression of a recessive phenotype when a recessive allele is present in a hemizygous state. A hemizygous state occurs when a locus is present on only one chromosome of a homologous pair. The recovery of recessive progeny in a cross of a homozygous-recessive or a heterozygous individual to a dominant individual with a known deletion locates the gene of interest to the deleted area. In some cases, however, single recessive alleles may have the dominant phenotype, and only the presence of two recessive alleles will produce the recessive phenotype (Burnham, 1962).

Chromosome Duplication. A duplication is a segment of a chromosome that is present more frequently than the remainder of the chromosome. A duplicate of a chromosome segment can occur at the end of or within a homologous or nonhomologous chromosome. It is produced by mutagenesis, is a regular occurrence in gametes produced by inversion or translocation heterozygotes, and occurs naturally.

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CHAPTER SIX

Quantitative Inheritance

The simultaneous segregation of many genes that control a quantitative character results in a range of genotypes that cannot be separated into distinct classes. Variation among individuals for a quantitative character also involves the effect of the environment on the phenotypic expression of a genotype. The study of the inheritance of quantitative characters is sometimes referred to as mathematical or statistical genetics because mathematical and statistical concepts are utilized.

CHARACTERIZATION OF A POPULATION

The evaluation of a quantitative character is based on the study of a population of genotypes. A population can be characterized for a trait by use of several different statistics (Falconer, 1981).

Population Mean

One important statistic used to describe a population is the mean performance of the genotypes it contains. A population of 10 genotypes with seed yields of 40, 41, 44, 46, 49, 50, 54, 58, 61, and 63 units would have a mean yield of 50.6 units. Effective selection among genotypes in a population results in a change in the mean population performance.

Falconer (1981) described the mean performance of a population for a single locus with two different alleles as

$$M = a(p - q) + 2dpq$$

where M = population mean
a = value of homozygous genotype
p = frequency of one allele

q = frequency of second allele
d = value of heterozygote

As an illustration of the components of the equation, consider a locus with two alleles, A_1 and A_2, that combine to form the genotypes A_1A_1, A_1A_2, and A_2A_2. The value of each genotype is designated with a symbol: $A_1A_1 = +a$, $A_2A_2 = -a$, and $A_1A_2 = d$. The value of a is the performance of a homozygous genotype minus the average performance of the two homozygous genotypes: $+a$ for $A_1A_1 = A_1A_1 - [(A_1A_1 + A_2A_2)/2]$ and $-a$ for $A_2A_2 = A_2A_2 - [(A_1A_1 + A_2A_2)/2]$. If $A_1A_1 = 20$ and $A_2A_2 = 14$, then $+a$ for $A_1A_1 = 20 - [(20 + 14)/2] = +3$ and $-a$ for $A_2A_2 = 14 - [(20 + 14)/2] = -3$.

The value of d is a measure of the degree of dominance between alleles. It is measured as the difference between the value of the heterozygote and the mean of the homozygotes.

$$d = A_1A_2 - \frac{A_1A_1 + A_2A_2}{2}$$

If the value of A_1A_2 is 19 and the average of the two homozygotes is 17, the value of d is $+2$. d is greater than zero but less than a with partial dominance, d equals a with complete dominance, and d is greater than a with overdominance.

The frequency of an allele in a population can vary from 0 to 1. The sum of the frequencies of alleles at a locus equals 1. For discussion of the population mean, the frequency of allele A_1 will be designated as p and the frequency of A_2 as q.

The values of a and d in a population do not change at a locus, but may vary among loci. Changes in the population mean are a result of changes in gene frequency.

The discussion thus far has dealt with a single locus. For a character controlled by many loci, the mean of a population is equal to the sum of the means for the individual loci, which can be expressed as

$$M = \Sigma\,[a(p - q) + 2dpq]$$

This expression assumes that there are no epistatic interactions between loci to influence the population mean (Falconer, 1981).

There is no way to measure the values of a, d, p, or q for individual loci of a polygenic character. Nevertheless, an understanding of their role in determining the population mean is helpful for evaluating the impact of selection on population performance.

Genotypic Values

A population can be characterized by the amount and type of genetic variability it contains. Genetic improvement of a quantitative character is based on effective selection among individuals that differ in genotypic value. The variation among

genotypic values represents the genotypic variance of a population. A description of the various types of gene action that determine the genotypic value of individuals in a population will be helpful in understanding the concept of genetic variance.

Genotypic value can be considered on the basis of a single locus or as a function of all loci of an individual that control a quantitative character (Falconer, 1981). The genotypic value of a single locus is equal to

$$G = A + D$$

where G is the genotypic value, A is the breeding value, and D is the dominance deviation. The genotypic value of all loci considered together is expressed as

$$G = A + D + I$$

where A is the sum of breeding values for separate loci, D is the sum of dominance deviations, and I is attributable to the interaction of alleles among loci, referred to as the interaction deviation or epistatic deviation. The type of gene action associated with the breeding value, dominance deviation, and epistatic deviation is an important concept in quantitative genetics.

Breeding Value. The breeding value of an individual is that portion of its genotypic value that determines the mean performance of its progeny (Falconer, 1981). The breeding value of an individual can be measured by mating the individual to a number of random individuals from a population, determining the mean performance of the progeny, subtracting the mean progeny performance from the population mean, and multiplying the deviation by two. The deviation is multiplied by two because half of the genes in the progeny are contributed by the individual and the other half are a random sample from the population with a value equal to the population mean.

The breeding value of an individual is determined by summation of the average effects of its genes, also referred to as the additive effect of genes. The average effect of a gene substitution is the regression coefficient (b) obtained from the linear regression of the genotypic value of an individual locus on the number of alleles of a certain type at that locus (Fisher, 1918,1941). If there is no dominance expressed at a locus, the linear regression line connects the genotypic values of the two homozygotes [Fig. 6-1(a)]. If dominance is expressed, none of the genotypic values will lie directly on the regression line [Fig. 6-1(b)].

Gene frequency in the population to which an individual is mated will influence the average effect of a gene substitution when dominance is present at a locus. If allele A_1 expresses some degree of dominance over A_2, the genotypic value of the heterozygote A_1A_2 will be closer to that of A_1A_1 than to that of A_2A_2. The effect of substituting A_2 for A_1 is greater when A_1A_2 is changed to A_2A_2 than when A_1A_1 is changed to A_1A_2. The average effect of a gene substitution depends, therefore, on the relative frequency of different genotypes in a population. Genotypic frequency in a population is determined by gene frequency.

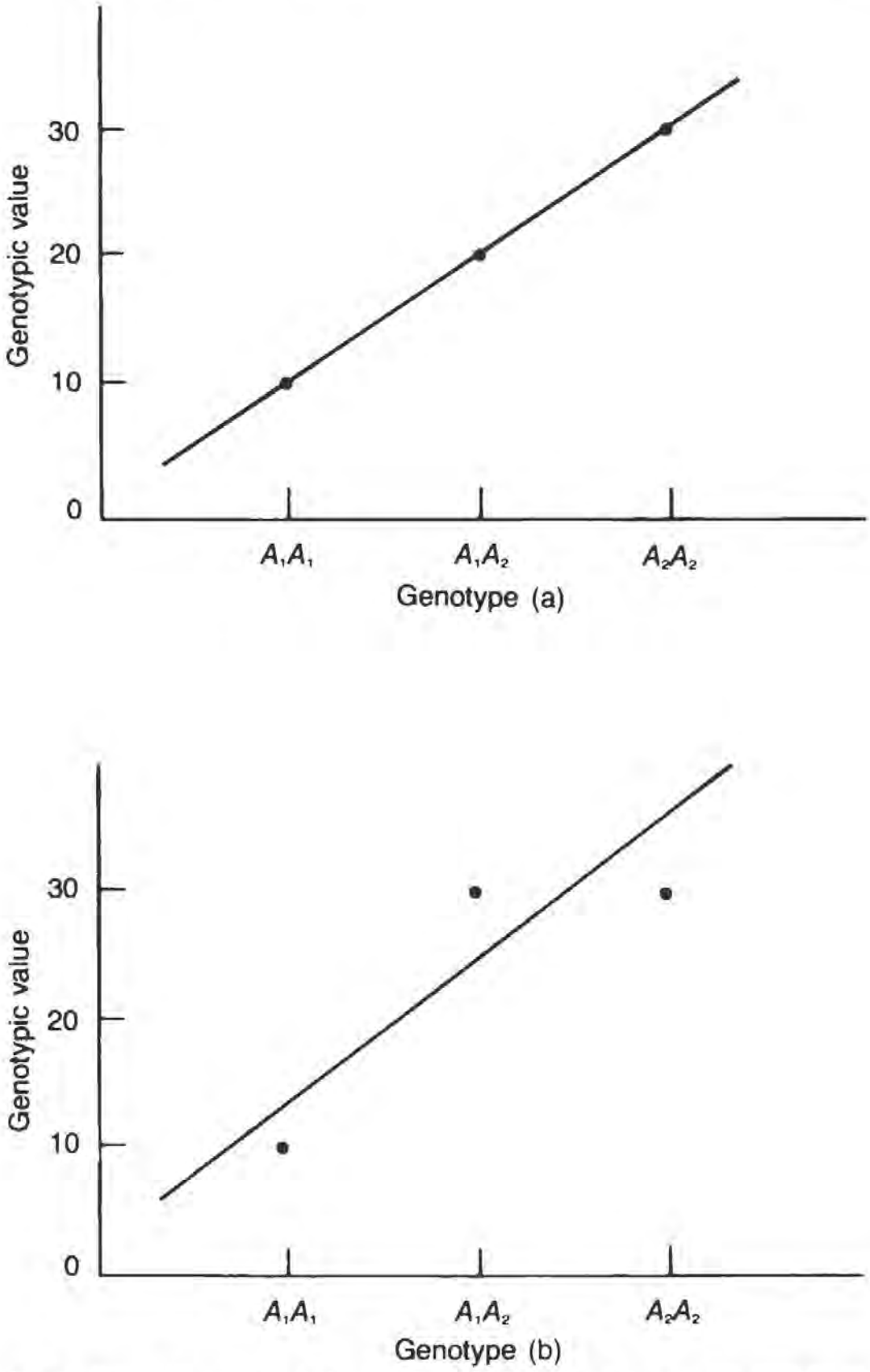


Figure 6-1 Regression of the genotypic value of a locus on the number of favorable alleles at that locus. (a) No dominance. (b) Complete dominance.

The relationship of the average effect of a gene substitution to the degree of dominance at a locus and gene frequency is summarized by the equation

$$\alpha = a + d(q - p)$$

where α = average effect of a gene substitution
a = difference between one homozygote and average of two homozygotes
d = difference between heterozygote and average of two homozygotes
q and p = frequency of alleles in population

The sum of the average effects of genes at all loci controlling a character determines the breeding value of an individual for that character. In the absence of epistasis, the sum of the average effects across loci is equal to the breeding value obtained by mating an individual to a population and measuring the deviation of progeny performance from the population mean.

Dominance Deviation. The dominance deviation (D) at a locus is the difference between the genotypic value (G) and the breeding value (A) of an individual, $D = G - A$ (Falconer, 1981). It represents the interaction of alleles at a locus, or intralocus interaction. The degree of dominance and gene frequency of a population influence genotypic values and breeding values; consequently, they also influence dominance deviations. The relationship among genotypic values, breeding values, and dominance deviations is illustrated in Fig. 6-2.

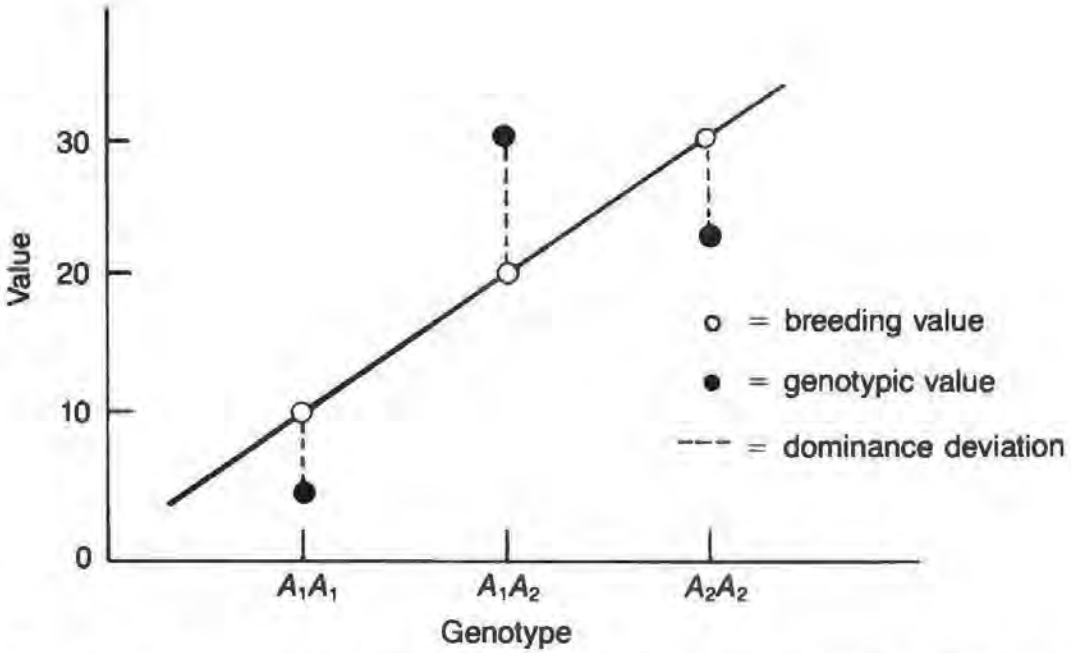


Figure 6-2 Relationship among genotypic value, breeding value, and dominance deviation.

Epistatic Interaction. The genotypic value of an individual for a quantitative character can be influenced by the interaction of alleles and genotypes at different loci, referred to as interlocus interaction. In the absence of epistasis, the genotypic value for all loci controlling a character is equal to the sum of the genotypic values for individual loci. When epistasis is present, the genotypic value for all loci is not accounted for completely by the genotypic values of individual loci.

The types of epistatic interaction described in quantitative genetics for two loci are additive × additive, additive × dominance, and dominance × dominance. For three loci, the number of possible interactions increases and includes additive × additive × additive, additive × additive × dominance, and so forth. In these expressions, "additive" refers to breeding values and "dominance" to dominance deviations. For two loci, additive × additive is the interaction of breeding values at both loci, additive × dominance is the interaction of breeding values at one locus and dominance deviations at the other, and dominance × dominance is the interaction of dominance deviations at both loci.

Epistatic interactions are dependent on the average effects of genes and dominance deviations at individual loci. As a result, they are dependent on the degree of dominance and the gene frequency in the population.

Types of Genotypic Variance

The genotypic variance among individuals in a population can be expressed as

$$\sigma_g^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$$

σ_g^2 represents the total genotypic variance (Falconer, 1981). σ_A^2, the additive variance, is the variation in breeding values among individuals. σ_D^2, the dominance variance, is the variation among individuals for dominance deviations. The epistatic variance, σ_I^2, is the variation associated with differences among individuals for epistatic interactions. The epistatic interaction can be subdivided further into variance components associated with the different types of interlocus interaction, such as additive × additive (σ_{AA}^2), additive × dominance (σ_{AD}^2), additive × additive × additive (σ_{AAA}^2), and so forth.

The magnitude of genetic variance components is unique to the population from which the components are obtained. Genotypic values, breeding values, dominance deviations, and epistatic interactions are influenced by the degree of dominance at a locus and the gene frequency of a population. Consequently, the variation among genotypic values is also a function of degree of dominance and gene frequency. This can be illustrated by considering the genotypic, additive, and dominance variances for one locus. The variance components are defined by the following equations (Falconer, 1981):

$$\sigma_A^2 = 2pq\,[a + d(q - p)]^2$$

$$\sigma_D^2 = (2pqd)^2$$

$$\sigma_g^2 = \sigma_A^2 + \sigma_D^2 = 2pq\,[a + d(q - p)]^2 + (2pqd)^2$$

Substitution of different values for d, p, and q will change the relative magnitudes of σ_A^2, $\frac{2}{D}$, and σ_g^2 (Falconer, 1981).

ESTIMATION OF GENETIC VARIANCES

There are a number of mating designs that can be used by the plant breeder to estimate the genotypic variance in a population. The mating designs differ in the genetic material evaluated, which determines the extent to which additive, dominance, and epistatic variances can be estimated.

A detailed review of alternative mating designs has been provided by Hallauer and Miranda (1981). Three of the more commonly used mating designs, the diallel, Design I, and Design II, will be used to illustrate the general procedure for estimating genotypic variances.

A number of criteria must be met for each mating design to obtain valid estimates of genotypic variance (Baker, 1978). Failure to meet one or more of these criteria may result in biased estimates of genotypic variance. A primary criterion for the diallel, Design I, and Design II mating designs is that individuals evaluated from a population be a random sample of all possible genotypes.

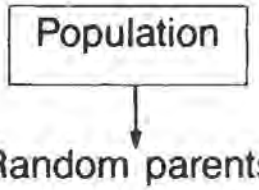
Diallel Design

The genetic material evaluated in the diallel mating design includes random individuals from a population and the progeny obtained by crossing those individuals in all combinations. The matings among individuals may include reciprocal crosses and selfed progeny.

To prepare the genetic material for the diallel, a group of random parents is identified in a population (Fig. 6-3). Self-pollinated seed of each genotype is maintained for evaluation of its performance per se. Each genotype is mated to every other genotype, and the seed from each mating is maintained separately. If the diallel is to include reciprocal crosses, each genotype is used as male and female for each mating and seed from the reciprocal crosses is kept separate.

The number of entries that are evaluated for the diallel mating design is determined by the number of genotypes (parents) sampled from the population. If the number of parents is designated as p, the number of pairwise matings among them is equal to $p(p - 1)/2$. Reciprocal crosses among pairwise combinations doubles the number of matings that must be made, expressed as $p(p - 1)$. If the number of genotypes is equal to 5, the number of pairwise matings is equal to $5(5 - 1)/2 = 10$, and the number of matings with reciprocal crosses is equal to $5(5 - 1) = 20$.

There are four combinations of crosses and selfed progeny of the parents that can be included for evaluation in the diallel mating design: (a) the crosses,



Single crosses without reciprocal matings or selfed progeny

	P_1	P_2	P_3
P_1	-	x	x
P_2	-	-	x
P_3	-	-	-

Single crosses with reciprocal matings

	P_1	P_2	P_3
P_1	-	x	x
P_2	x	-	x
P_3	x	x	-

Single crosses with reciprocal matings and selfed progeny

	P_1	P_2	P_3
P_1	⊗	x	x
P_2	x	⊗	x
P_3	x	x	⊗

Figure 6-3 Derivation of progenies in a diallel mating design. Each parent is mated with every other parent, and reciprocal matings and selfed progeny also can be evaluated.

Table 6-1 Analysis of Variance of Diallel Crosses for $n(n-1)/2$ Crosses of n Parents Where Expected Mean Squares, E(MS), Are Expressed in Terms of Covariances of Relatives

Source	Degrees of Freedom	Mean Squares	Expected Mean Squares
Replications	$r-1$		
Crosses	$[n(n-1)/2]-1$	M_2	$\sigma^2 + r\sigma_c^2$
GCA	$n-1$	M_{21}	$\sigma^2 + r(\text{Cov FS} - 2\text{ Cov HS}) + r(n-2)\text{Cov HS}$
SCA	$n(n-3)/2$	M_{22}	$\sigma^2 + r(\text{Cov FS} - 2\text{ Cov HS})$
Error	$(r-1)\{[n(n-1)/2]-1\}$	M_1	σ^2
Total	$rn-1$		

GCA = general combining ability; SCA = specific combining ability; r, n = number of replications and parents, respectively; M = mean squares; Cov FS, Cov HS = covariance of full-sib families and of half-sib families, respectively.

Source: Hallauer and Miranda, 1981.

(b) the crosses and selfed progeny of the parents, (c) the reciprocal crosses, and (d) the reciprocal crosses and selfed progeny of the parents (Griffing, 1956).

The separation of the genotypic variance that is possible with the diallel cross can be illustrated with an analysis of variance that involves only the crosses (Table 6-1). The variation among crosses in the diallel can be divided into variation among half-sib families and variation among full-sib families. There is one half-sib family for each parent in a diallel. The performance of a half-sib family is determined by averaging the performance of all crosses with one parent in common. The variation among the half-sib families in a diallel is an estimate of general combining ability (GCA). A full-sib family is the mating of two parents; therefore, the number of full-sib families in a diallel equals the number of single crosses that are evaluated. The performance of the full-sib families is used to obtain an estimate of specific combining ability (SCA).

The genetic variance components associated with the covariance of half-sib families (Cov HS) and full-sib families (Cov FS) depends on the inbreeding (F) of the genotypes used as parents in the diallel. When the parents are F_2 or S_0 plants or lines derived from them (F = 0), the genetic variance components are

$$\text{Cov HS} = \tfrac{1}{4}\sigma_A^2 + \tfrac{1}{16}\sigma_{AA}^2 + \text{higher orders of additive epistasis}$$

$$\text{Cov FS} = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_D^2 + \tfrac{1}{4}\sigma_{AA}^2 + \text{other forms of additive and dominance epistasis}$$

Assuming there is no epistasis, the Cov HS is multiplied by four to obtain σ_A^2; $\sigma_A^2 = \frac{1}{4}\sigma_A^2 \times 4$. An estimate of σ_D^2 is obtained as

$$\begin{aligned}\sigma_D^2 &= 4\,(\text{Cov FS} - 2\,\text{Cov HS}) \\ &= 4[(\tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_D^2) - 2(\tfrac{1}{4}\sigma_A^2)]\end{aligned}$$

When the parents are random inbred lines from a population (F = 1), the genetic variance components are

$$\text{Cov HS} = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_{AA}^2 + \text{higher orders of additive epistasis}$$

$$\text{Cov FS} = \sigma_A^2 + \sigma_D^2 + \sigma_{AA}^2 + \text{higher orders of additive and dominance epistasis}$$

Assuming there is no epistasis, the Cov HS is multiplied by two to obtain σ_A^2; $\sigma_A^2 = \frac{1}{2}\sigma_A^2 \times 2$. An estimate of σ_D^2 is obtained as

$$\text{Cov FS} - 2\,\text{Cov HS} = (\sigma_A^2 + \sigma_D^2) - 2(\tfrac{1}{2}\sigma_A^2)$$

Design I (Nested Design)

Genotypic variance can be subdivided into components by a mating design referred to as Design I or the nested design. Genetic material for this mating

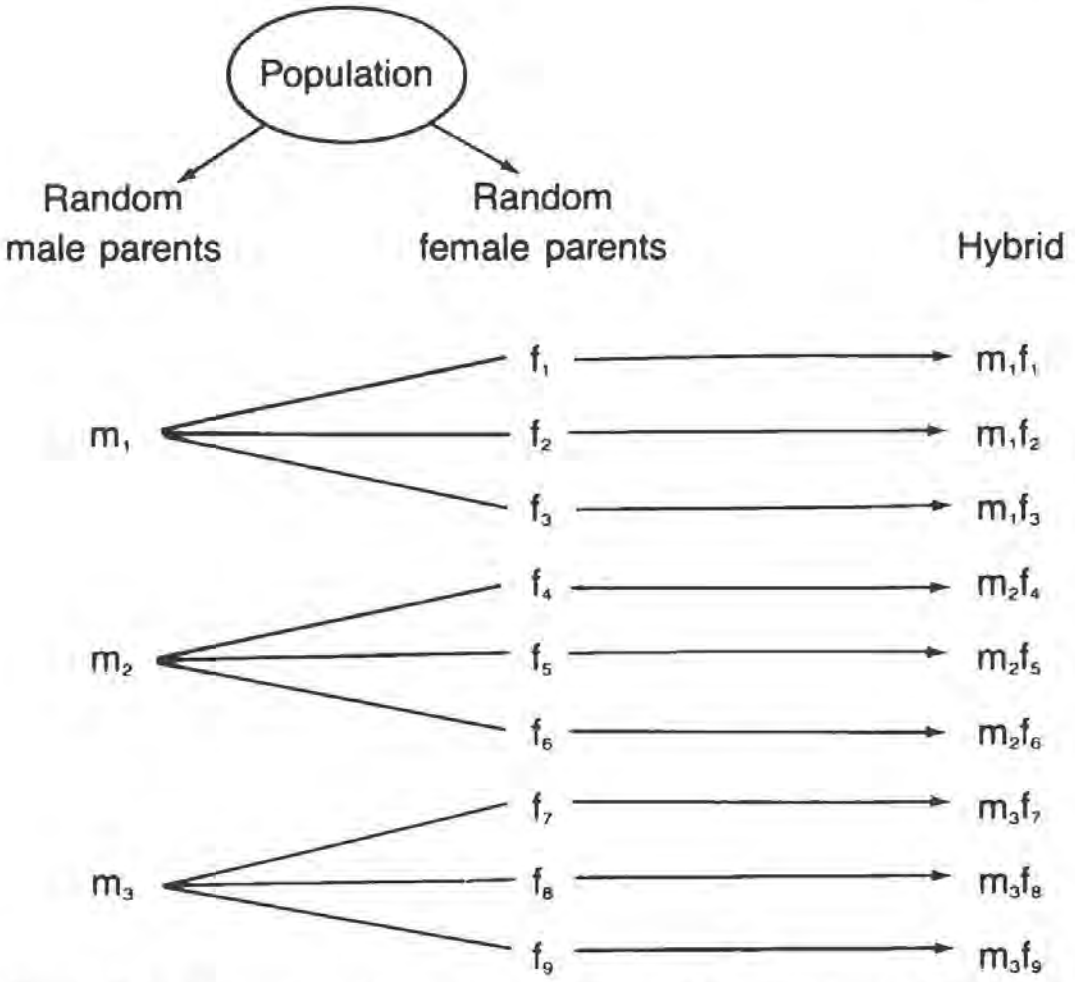


Figure 6-4 Derivation of progenies in a Design I mating design. Each male parent is crossed to different female parents from the population.

design involves crosses among random parents from a population, some of which are designated as males and others as females. Each male plant is mated to an equal number of females (Fig. 6-4). A different group of female parents is used for each male. The number of single crosses formed by the matings is equal to the number of males (p_m) times the number of females (p_f) mated to each male: $p_m \times p_f$. If 10 male parents are each mated to 5 female parents, 50 single crosses are evaluated.

The variation among single crosses is divided into variation among males and variation among females within males (Table 6-2). The expected mean squares for the sources of variation include the covariance of half-sibs (Cov HS) and the covariance of full-sibs (Cov FS). The genetic variance components associated with these covariance terms and their use to obtain estimates of σ_A^2 and σ_D^2 are the same as described for the diallel design (Hallauer and Miranda, 1981).

Table 6-2 Analysis of Variance of Design I Mating Design for One Environment

Source	Degrees of Freedom	Mean Squares	Expected Mean Squares	
			Components of Variance	Covariances of Relatives
Replications	$r-1$			
Males	$m-1$	M_3	$\sigma^2 + r\sigma^2_{f/m} + rf\sigma^2_m$	$\sigma^2 + r(\text{Cov FS} - \text{Cov HS}) + rf\text{Cov HS}$
Females/males	$m(f-1)$	M_2	$\sigma^2 + r\sigma^2_{f/m}$	$\sigma^2 + r(\text{Cov FS} - \text{Cov HS})$
Error	$(r-1)(mf-1)$	M_1	σ^2	σ^2
Total	$rmf-1$			

r, m, and f = number of replications, males, and females within males, respectively; M = mean squares; Cov FS, Cov HS = covariance of full-sib and half-sib families, respectively.

Source: Hallauer and Miranda, 1981.

Design II (Factorial Design)

Design II is a factorial mating design in which some parents from a population are designated as male and others as female. Each male parent is mated to each female, but male parents are not crossed to each other and female parents are not crossed to each other (Fig. 6-5). The number of single crosses included is equal to the number of male parents (p_m) times the number of female parents (p_f) : $p_m \times p_f$. If there were eight male and six female parents, 48 single crosses would be included in the experiment.

Variation among crosses is divided into variation among male parents, variation among female parents, and the interaction of male and female parents (Table 6-3). The covariance among half-sib families can be designated as Cov HS_m when the male parent is common to all crosses and Cov HS_f when the female parent is common to all crosses. The genetic variance components associated with the two covariances are the same. When the parents are noninbred (F = 0),

$$\text{Cov } HS_m \text{ and Cov } HS_f = \tfrac{1}{4}\sigma_A^2 + \tfrac{1}{16}\sigma_{AA}^2 + \text{other forms of additive epistasis}$$

When the parents are inbred (F = 1)

$$\text{Cov } HS_m \text{ and Cov } HS_f = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_{AA}^2 + \text{other forms of additive epistasis}$$

The separate covariances for half-sib families of male and female parents provide separate estimates of σ_A^2. An estimate of σ_D^2 is obtained from the relationship Cov FS − (Cov HS_m + Cov HS_f).

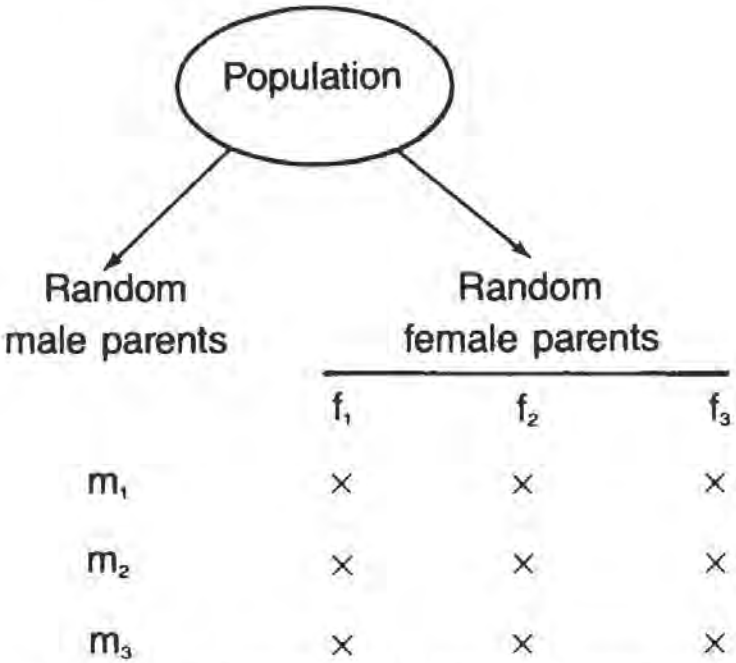


Figure 6-5 Derivation of progenies in a Design II mating design. The male parents are each crossed to the same female parents.

Table 6-3 Analysis of Variance of Design II Mating Design for One Environment

Source	Degrees of Freedom	Mean Squares	Expected Mean Squares	
			Components of Variance	Covariances of Relatives
Replications	$r-1$			
Males	$m-1$	M_4	$\sigma^2 + r\sigma^2_{fm} + rf\sigma^2_m$	$\sigma^2 + r(\text{Cov FS} - \text{Cov HS}_f - \text{Cov HS}_m) + \text{rfCov HS}_m$
Females	$f-1$	M_3	$\sigma^2 + r\sigma^2_{fm} + rm\sigma^2_f$	$\sigma^2 + r(\text{Cov FS} - \text{Cov HS}_f - \text{Cov HS}_m) + \text{rmCov HS}_f$
Males × females	$(m-1)(f-1)$	M_2	$\sigma^2 + r\sigma^2_{fm}$	$\sigma^2 + r(\text{Cov FS} - \text{Cov HS}_f - \text{Cov HS}_m)$
Error	$(r-1)(mf-1)$	M_1	σ^2	σ^2
Total	$rmf-1$			

r, m, and f = number of replications, males, and females, respectively; M = mean squares; Cov FS and Cov HS = covariance of full-sib and half-sib families, respectively.

Source: Hallauer and Miranda, 1981.

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CHAPTER SEVEN

Heritability

The effectiveness of selection for a trait depends on the relative importance of genetic and nongentic factors in the expression of phenotypic differences among genotypes in a population, a concept referred to as heritability. The heritability of a character has a major impact on the methods chosen for population improvement, inbreeding, and other aspects of selection. Single-plant selection may be effective for a character with high heritability and relatively ineffective for one with low heritability. The extent to which replicated testing is required for selection will depend on the heritability of the character.

COMPONENTS OF HERITABILITY

Heritability (h^2) can be defined as the ratio of the genotypic variance (σ_g^2) to the phenotypic variance (σ_{ph}^2); $h^2 = \sigma_g^2 / \sigma_{ph}^2$. The phenotypic variance can be subdivided into components of variance attributable to factors that cause differences in the performance among individuals. This relationship can be expressed as $\sigma_{ph}^2 = \sigma_e^2 + \sigma_{ge}^2 + \sigma_g^2$. The variance component σ_e^2 is a measure of differences among phenotypes caused by the failure to treat each genotype exactly alike, generally referred to as experimental error or environmental variance. σ_{ge}^2 represents differences among phenotypes that are caused by genotype × environment interaction, which is the failure of genotypes to perform the same relative to each other when they are evaluated in different locations and/or years. σ_{ge}^2 is the sum of genotype × location (σ_{gl}^2), genotype × year (σ_{gy}^2), and genotype × location × year (σ_{gly}^2) interaction. Genotype × environment interaction is discussed in more detail in Chap. 18. The genotypic variance, σ_g^2, is the variation caused by genetic differences among individuals. The genotypic variance is the sum of the additive (σ_A^2), dominance (σ_D^2), and epistatic (σ_I^2) variances (Chap. 6).

TYPES OF HERITABILITY

Heritability can be expressed in a broad sense or a narrow sense. Broad-sense heritability is a ratio of the total genotypic variance, including additive, dominance, and epistatic variance, to the phenotypic variance, $\sigma_g^2/\sigma_{ph}^2 = (\sigma_A^2 + \sigma_D^2 + \sigma_I^2)/\sigma_{ph}^2$. Heritability in the narrow sense is a ratio of the additive genetic variance to the phenotypic variance, σ_A^2/σ_{ph}^2. Narrow-sense heritability is the more useful concept because it measures the relative importance of the additive portion of the genetic variance that can be transmitted to the next generation of offspring. This is particularly important when heritability is used to predict gain expected from selection for a character (Chap. 17).

The heritability of a character is not a constant value. Decisions made by the breeder can influence the magnitude of heritability and the amount of genetic improvement obtained from selection. An understanding of the factors that contribute to heritability permits the breeder to develop a breeding program that maximizes genetic improvement with available resources. Because many factors can influence heritabilities, estimates of them should be interpreted with regard to the conditions under which they were obtained.

FACTORS INFLUENCING THE MAGNITUDE OF HERITABILITY ESTIMATES

Population Characterized

Estimates of heritability are influenced by the amount of genotypic variance present for a trait in the population being studied. The number and genetic diversity of parents used to form a population will have a direct bearing on the amount of genetic variation present. A population derived from crosses between many divergent parents is expected to express more genetic variance than a population derived from a few related parents.

The amount of self-pollination in a population will influence the genetic variance among individuals. As the level of inbreeding increases, the magnitude of the genetic variance among individuals increases. Consequently, the heritability estimate obtained from evaluation of F_2 plants can differ from an estimate obtained with F_4 individuals.

Sample of Genotypes Evaluated

Heritability estimates are obtained by evaluating a relatively small number of individuals in a population. If all possible segregates of a population could be evaluated, the true genetic variance of a population could be determined. The number of possible segregates in a population is so large, however, that it is impossible to evaluate them all. For example, the number of possible genotypes

in a diploid species for a character controlled by 10 independent loci with two alleles is $3^{10} = 59{,}049$. Considering that important quantitative characters such as yield are probably controlled by more than 10 loci, only a sample of possible genotypes in a population can be measured. The genetic variance obtained from a sample of individuals is an estimate of the true genetic variance in the population. Consequently, the heritability computed for a sample of genotypes is an estimate of the true heritability of a population.

The relationship between a heritability estimate obtained from a sample of genotypes and the true heritability of a population depends on the manner in which the sample is chosen for evaluation. If the genotypes are chosen at random from all possible members of the population, the genetic variance among the random genotypes is considered a valid estimate of the true genetic variance of the population, and the heritability estimate is considered a valid estimate of the true heritability. Stated in another manner, results obtained with random genotypes are considered applicable to all members of the population.

Valid estimates of the genetic variance and the heritability of a trait cannot be obtained when genotypes are purposely selected from a population rather than chosen at random. Consider a population that has extensive variation for plant height, some of the plants being shorter and others taller than would be acceptable in a cultivar. If the short and tall plants intentionally are not included in a sample obtained from the population, the genetic variance and heritability estimates for plant height obtained with the selected sample would not be representative of the whole population.

When genotypes are a nonrandom sample, results obtained from their evaluation pertain only to those genotypes and cannot be used to infer what would be expected if random genotypes were studied. A breeder may choose to evaluate the genetic variation among selected genotypes and compute the ratio of genetic variation to the phenotypic variation. The ratio is not referred to as heritability. The term repeatability has been used when a nonrandom sample of genotypes is evaluated. It should be noted that repeatability used in this sense has a different meaning than when used to describe the correlation between repeated measurements on the same individual, as discussed by Falconer (1981).

Heritability also has been used to describe the ratio of genotypic to phenotypic variance among random genotypes that are not part of a segregating population. Assume there is a quantitative character for which cultivars of a species have not been evaluated previously. A random group of the cultivars is evaluated, the genotypic and phenotypic variances among them are determined, and a ratio of genotypic to phenotypic variance is computed. In this context, the reference population is cultivars of the species, not a segregating population.

Method of Calculation

The heritability of a character can be computed by a number of methods. The values obtained by different methods will vary to some extent.

Variance Component Method. The method that provides the greatest flexibility for predicting the effectiveness of alternative selection procedures is based on variance components obtained from analysis of variance procedures (Table 7-1). This method is discussed in the chapters dealing with maximizing genetic improvement (Chap. 17) and with genotype × environment interaction (Chap. 18). The components of variance can be used to compute heritability estimates on a single plant, a plot, or an entry-mean basis.

The calculation of heritability in a narrow sense requires an estimate of the additive genetic variance in a population. Several mating designs are available to obtain such estimates, including the diallel, design I, and design II (Chap. 6).

Parent-Offspring Regression. The linear regression of the performance of offspring on that of the parents was proposed by Lush (1940) as a method of estimating heritability. The linear regression model is

$$Y_i = a + bX_i + e_i$$

Table 7-1 Equations for Computing Heritabilities by the Variance Component Method with Four Different Selection Units

Heritability on single-plant basis when selection is based on plants of a population that are not subdivided into plots or blocks

$$h^2 = \frac{\sigma_g^2}{\sigma_w^2 + \sigma^2 + \sigma_{ge}^2 + \sigma_g^2}$$

Heritability on single-plant basis when selection is based on comparison of plants within a plot. Formula also applies when plants of a population are divided into blocks of a grid and the plants within a block are compared with each other without regard to performance of plants in other blocks (Gardner, 1961).

$$h^2 = \frac{\sigma_g^2}{\sigma_w^2 + \sigma_{ge}^2 + \sigma_g^2}$$

Heritability on plot basis

$$h^2 = \frac{\sigma_g^2}{\sigma_w^2/n + \sigma^2 + \sigma_{ge}^2 + \sigma_g^2} = \frac{\sigma_g^2}{\sigma_e^2 + \sigma_{ge}^2 + \sigma_g^2}$$

Heritability on entry-mean basis

$$h^2 = \frac{\sigma_g^2}{\sigma_e^2/rt + \sigma_{ge}^2/t + \sigma_g^2}$$

h^2 = heritability; σ_g^2 = genetic variance; σ_w^2 = variance among plants within a plot; σ^2 = variance among plots or blocks; σ_e^2 = experimental error = $(\sigma_w^2/n) + \sigma^2$; σ_{ge}^2 = genotype × environment interaction; n = number of plants within a plot or block; r = number of replications; t = number of test environments.

where Y_i = performance of offspring of the ith parent

a = mean performance of all parents evaluated

b = linear regression coefficient

X_i = performance of ith parent

e_i = experimental error associated with measurement of X_i

In plant species "parent" refers to a random plant or a line from a population, and "offspring" are half-sib or selfed progeny. A mid-parent–offspring regression also can be used, which is the relationship between the average performance of two parents and their full-sib offspring.

The relationship of the regression coefficient to heritability depends on the type of offspring that is evaluated. The type of offspring also determines if a narrow- or broad-sense heritability is obtained. As an illustration of these principles, consider the evaluation of F_2 plants from a random-mating population with no inbreeding. The evaluation of half-sib progeny from F_2 plants will be considered first. The half-sib seed is obtained by mating an F_2 plant (parent) to a random sample of gametes from F_2 plants in the population. Half of the alleles in the offspring will be from the parent and half from the population. The value of b obtained from the regression of half-sib offspring on their parents is equal to $^1/_2$ the heritability value. The b value is multiplied by two to obtain the heritability estimate, $2b = h^2$.

The equation for the linear regression coefficient is $b = \sigma xy/\sigma_x^2$, where σ^{xy} is the covariance between parents (x) and their offspring (y) and σ_x^2 is the phenotypic variation among the parents. The genetic relationship defined by the covariance determines if the numerator includes only additive genetic variance for narrow-sense heritability or if other types of genetic variance are present for broad-sense heritability. For the covariance of half-sib offspring on their parents, the genetic components include additive variance and additive forms of epistasis, but no dominance. The heritability can be considered narrow-sense unless the amount of additive epistasis is important.

A second type of offspring from F_2 plants is selfed progeny. Random F_2 plants are measured, selfed seed is obtained from each, and the selfed offspring are evaluated. All of the alleles in the offspring are obtained from the parent. The b value obtained from the parent-offspring regression is equal to the heritability, $b = h^2$. The genetic components defined by the covariance of parent and offspring include additive, dominance, and epistasis; therefore, broad-sense heritability is obtained.

A third type of offspring is the full-sib progeny obtained by mating two random F_2 plants. The two F_2 plants of a mating are measured and their average or mid-parent value is determined. The performance of the full-sib progeny is regressed on the mid-parent value. The alleles in the full-sib offspring are obtained from the two parents of a mating, and the regression coefficient is equal to the heritability, $b = h^2$. Additive and additive forms of epistasis are included

in the covariance; therefore, narrow-sense heritability is obtained by mid-parent–offspring regression when additive epistasis is not important.

Use of parent-offspring regression is based on several assumptions: (a) The character of interest has diploid Mendelian inheritance, (b) the population is random-mated, (c) the population is in linkage equilibrium or there is no linkage among loci controlling a character, (d) parents are noninbred, and (e) there is no environmental correlation between the performance of parents and offspring (Vogel et al., 1980). Failure to meet the assumptions can bias the heritability estimates obtained. When the parents are inbred, an adjustment factor can be applied to the heritability estimate (Smith and Kinman, 1965). Environmental correlation between the performance of parents and offspring generally is not a problem when parents and offspring are randomized as independent entries in an experiment. The assumption may not be met if parents and progeny are evaluated in the same plot instead of being randomized within replications (Vogel et al., 1980).

Parent-Offspring Correlation. Correlation of the performance of a parent with that of its offspring was proposed by Frey and Horner (1957) as an alternative to the parent-offspring-regression method for computing heritability. When parents are measured in one season and their offpsring in another, environmental differences between the two seasons can cause the range in phenotypes among the parents to be greater or less than that for the offspring. As a result, heritability percentages obtained by parent-offspring regression could have maximum values greater than 100 percent. To eliminate this effect of environment, the use of standard unit heritabilities obtained by calculating parent-offspring regressions on data coded in terms of standard deviation units was suggested. Such a procedure leads to results equivalent to the coefficient obtained from a simple parent-offspring correlation.

Indirect Estimates of Environmental Variation. A method was proposed by Mahmud and Kramer (1951) to estimate broad-sense heritability on a single-plant basis. The method involves the measurement of a character on F_2 plants of a single-cross population and on the inbred parents used to form the population. The formula these investigators presented for estimating heritability is

$$h^2 = \frac{\sigma^2_{F2} - \sqrt{(\sigma^2_{P1})(\sigma^2_{P2})}}{\sigma^2_{F2}}$$

where h^2 = heritability

σ^2_{F2} = phenotypic variance among F_2 plants

σ^2_{P1} and σ^2_{P2} = phenotypic variances among plants of parents of single-cross population

σ^2_{F2} includes the additive, dominance, and epistatic genetic variance, variation due to genotype × environment interaction, and variation due to environmental effects (experimental error). The environmental variation is estimated by the variation among plants of the inbred parents that are considered genetically homogeneous. The difference between the variance among the F_2 plants and the geometric mean of the variance for the parents is considered an estimate of the genetic variance (σ^2_g).

The variation among genetically homogeneous F_1 plants obtained from a cross between inbred parents also can provide an estimate of environmental variation, in addition to that obtained from the parents. The numerator of the equation becomes

$$\sigma^2_{F2} - \sqrt[3]{(\sigma^2_{P1})(\sigma^2_{P2})(\sigma^2_{F1})}$$

One potential weakness of the method is that environmental variation among F_2 plants may not be equivalent to that of the parents or of the F_1. For species that are subject to extensive inbreeding depression, weak inbred plants may be subject to more environmental variation than vigorous F_2 plants. When heterosis is large, hybrid F_1 plants may be less sensitive to environmental fluctuations than are F_2 plants (Warner, 1952).

Backcross Method. A method for estimating narrow-sense heritability on a single-plant basis was developed by Warner (1952). It involves the measurement of F_2 plants from a cross between inbred parents and F_2 plants from populations developed by backcrossing the single-cross hybrid to each of the inbred parents. The formula used to compute heritability is

$$h^2 = \frac{2(\sigma^2_{F2}) \quad (\sigma^2_{B1} + \sigma^2_{B2})}{\sigma^2_{F2}}$$

where σ^2_{F2} is the variance among F_2 plants of the single-cross population and σ^2_{B1} and σ^2_{B2} are the variances among F_2 plants from the backcrosses of the single-cross F_1 to parent 1 and parent 2. The numerator of the equation represents additive genetic variance, and σ^2_{F2} in the denominator represents the phenotypic variance among plants.

Realized Heritability. The heritability of a character can be estimated by the amount of genetic improvement that is realized by selection within a population (Falconer, 1981). The general formula used is $h^2 = R/S$, where R is the response realized by selection and S is the selection differential. The selection differential is the difference between the mean of individuals selected from a population and the overall mean of the population from which they were selected.

The method can be illustrated by considering the performance of F_2 plants and their F_3 progeny. The mean performance of all F_2 plants can be designated

$\bar{x}_{F2}$, and the mean of selected F_2 plants as $\bar{x}_{s,F2}$. The selection differential in F_2 is

$$S = \bar{x}_{s,F2} - \bar{x}_{F2}$$

The mean performance of all F_3 progeny from F_2 plants can be designated $\bar{x}_{F3}$ and the mean of F_3 progeny from selected F_2 plants as $\bar{x}_{s,F3}$. The realized response from selection is $R = \bar{x}_{s,F3} - \bar{x}_{F3}$. Realized heritability can be summarized as

$$h^2 = \frac{\bar{x}_{s,F3} - \bar{x}_{F3}}{\bar{x}_{s,F2} - \bar{x}_{F2}}$$

Realized heritability can be computed on a single-plant, a plot, or an entry-mean basis. The basis of the heritability depends on the unit used for selection. In the previous illustration, selection among F_2 plants provided an estimate of heritability on a single-plant basis.

An alternative procedure for computing realized heritability involves selection within a population for individuals with high or low values for a trait. The progeny of individuals in each group are evaluated. Realized heritability is expressed as the difference in mean performance of high and low progeny divided by the difference in the mean of the parents. If F_2 plants and their progeny are evaluated, the equation can be expressed as

$$h^2 = \frac{\bar{x}_{high,F3} - \bar{x}_{low,F3}}{\bar{x}_{high,F2} - \bar{x}_{low,F2}}$$

where $\bar{x}_{high,F3}$ = mean performance of F_3 progeny of F_2 plants selected in high group

$\bar{x}_{low,F3}$ = mean performance of F_3 progeny of F_2 plants in low group

$\bar{x}_{high,F2}$ = mean performance of F_2 plants in high group

$\bar{x}_{low,F2}$ = mean performance of F_2 plants in low group

Realized heritability may not provide a valid estimate of the true heritability of a population (Falconer, 1981). Changes in the population unrelated to selection could bias the heritability estimates. These could include systematic changes due to environmental trends, inbreeding depression, and random drift.

Extensiveness of Genotype Evaluation

Selection among genotypes in a plant species can be based on the performance of single plants or on the average performance of progeny of a genotype evaluated in one or more replications, locations, and years. The heritability of a character and the effectiveness of selection are a function of the extensiveness with which

a genotype is evaluated. The heritability of a character may be relatively low when individual plants are evaluated for selection and relatively high when individuals are selected on the basis of the average performance of their progeny when tested in multiple environments. The most useful descriptions of the heritability of a character include information on the extensiveness with which genotypes were evaluated.

Heritability is commonly described on a single plant, a plot, or an entry-mean basis. Heritability on a single-plant basis can be described for selection among or within plots. The heritabilities differ in the extent to which factors other than the additive genetic variance among genotypes contribute to the phenotypic variance. This can be illustrated by examining the equations that are used to compute heritability by the variance component method (Table 7-1).

Heritability is lowest for the selection among single plants in a population that is not subdivided into plots. When single plants within a plot are compared, plot-to-plot variation (σ^2) does not contribute to the phenotypic variation (Hallauer and Miranda, 1981) (Chap. 17). The phenotypic variation on a plot basis is affected by the number of plants per plot whose measurements are averaged to obtain a single value for the character. The largest amount of influence that a breeder can have on the phenotypic variation is for heritability on an entry-mean basis. It is influenced by number of plants per plot, replications, locations, and years over which a genotype is evaluated.

Failure to estimate genotype × location (σ^2_{gl}), genotype × year, (σ^2_{gy}) and genotype × location × year (σ^2_{gly}) interactions can result in an inflated estimate of the genetic variance and heritability of a character (Chap. 18). The genotype variance, σ^2_g, can only be separated from the three interactions when genotypes are tested in two or more locations and years. If genotypes are evaluated at only one location in one year, σ^2_g cannot be separated from the three interactions and an estimate of heritability is equal to

$$\frac{\sigma^2_g + \sigma^2_{gl} + \sigma^2_{gy} + \sigma^2_{gly}}{\sigma^2_{ph}}$$

A heritability estimate based on data from one location in two or more years would be $(\sigma^2_g + \sigma^2_{gl})/\sigma^2_{ph}$, and an estimate derived from one year of data at two or more locations would be $(\sigma^2_g + \sigma^2_{gy})/\sigma^2_{ph}$. If the interactions with genotypes are important, heritability estimates will be inflated whenever genotypes are evaluated at less than two locations in two years.

Linkage Disequilibrium

Two alleles at each of two loci can be linked in coupling $\frac{AB}{ab}$ or repulsion $\frac{Ab}{aB}$. A population is said to be in linkage disequilibrium when the frequency of

coupling and repulsion phase linkages are not equal. Linkage disequilibrium can influence heritability estimates by causing an upward bias (increase) or downward bias (decrease) in the estimates of additive and dominance genetic variance. An upward bias in the additive variance (σ_A^2) will inflate the heritability, and downward bias will cause the heritability to be lower than if linkage equilibrium were present.

An excessive frequency of coupling phase linkages causes an upward bias in the estimates of additive and dominance variances (Hallauer and Miranda, 1981). When an excess of repulsion phase linkages is responsible for the disequilibrium, there is an upward bias in the dominance variance and a downward bias in the additive variance.

Linkage disequilibrium can be reduced by random-mating of a population. The number of generations of hybridization required to achieve linkage equilibrium depends on the closeness of the linkage (Hanson, 1959).

Conduct of Experiment

The accuracy with which measurements of the character(s) of interest can be made will affect experimental error. The degree of uniformity in the test environment will also influence the experimental error. Any precautions that the breeder can take to reduce experimental error (σ_e^2) will improve the heritability of a character (Table 7–1).

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CHAPTER EIGHT

Inbreeding

Inbreeding represents the mating of individuals that are more closely related than individuals mated at random in a population. The most extreme form of inbreeding is the mating of an individual to itself by self-pollination. Less extreme forms of inbreeding also play an important role in plant breeding.

CONSEQUENCES OF INBREEDING

The mating of related individuals increases homozygosity by bringing together identical alleles at a locus. Homozygosity permits the expression of recessive alleles that may have been masked by a dominant allele in the parents. When the recessive alleles are less favorable than dominant ones, the overall desirability of individuals decreases. This reduction in performance is referred to as inbreeding depression.

There are major differences among species for the amount of inbreeding depression that is expressed. In self-pollinated species such as wheat and oat, inbreeding depression is minimal and homozygous genotypes are used as cultivars for crop production. In some cross-pollinated diploid species such as maize, homozygous genotypes can be produced readily, but their performance is lower than that of the hybrid cultivars used in commercial production. Inbreeding depression is so severe in some cross-pollinated polyploid species, such as alfalfa, that homozygous genotypes do not survive.

PURPOSES OF INBREEDING

One important purpose of inbreeding is the development of genotypes that can be maintained through multiple generations of seed production. Self-pollinated

cultivars are reproduced for many generations without changes in their genetic composition. Reliable production of inbred commercial hybrid seed of a species is dependent on the availability of inbred parents whose genotype can be maintained.

Inbreeding has been used to reduce the frequency of deleterious recessive alleles in genotypes that serve as parents of a synthetic or a vegetatively propagated cultivar. One selfed generation may permit the expression and subsequent elimination of deleterious alleles without excessive inbreeding depression occurring in lines selected for use as parents for population development.

Inbreeding increases the genetic variability among individuals in a population. Greater genetic variability among inbred progeny can increase the effectiveness of selection and the amount of genetic improvement in a breeding program (Chap. 17).

INBREEDING IN DIPLOID SPECIES

The degree of relationship among individuals was described by Wright (1921,1922) as the coefficient of inbreeding (F). Later, Malecot (1948) defined the coefficient of inbreeding as the probability that two alleles at a locus are identical by descent. The value of F for a population describes the average level of homozygosity present.

A close relationship has been found in diploid species between the coefficient of inbreeding and the degree of inbreeding depression. A review of experimental data in maize led Hallauer and Miranda (1981) to conclude that (a) there was a linear relationship between percentage of homozygosity and performance of quantitative characters, (b) the method of inbreeding made no practical difference in the performance of lines at a comparable level of inbreeding, (c) the decrease in performance associated with a reduction in the level of heterozygosity was adequately described by the additivity of unlinked loci, and (d) the effects of epistasis on inbreeding depression did not seem to be important.

Four mating systems used to increase homozygosity in a breeding population are self-pollination, full-sib mating, half-sib mating, and backcrossing (Fig. 8-1). Self-pollination occurs when male and female gametes from the same individual unite to produce seed. Full-sib mating represents the crossing of pairs of individuals in a population. Half-sib mating occurs when individual plants are fertilized by random pollen from the population. Backcrossing is a mating scheme in which individuals in a population are crossed to one of their parents in successive generations.

The changes in homozygosity (F) with different inbreeding methods in a diploid species are calculated in Table 8-1. The F value for the F_2 generation is defined as 0. The percentage of homozygosity associated with each generation of backcrossing depends on the level of inbreeding of the recurrent parent. A noninbred recurrent parent ($F = 0$) refers to an F_2 population or its equivalent, and an inbred recurrent parent ($F = 1$) is a completely homozygous individual.

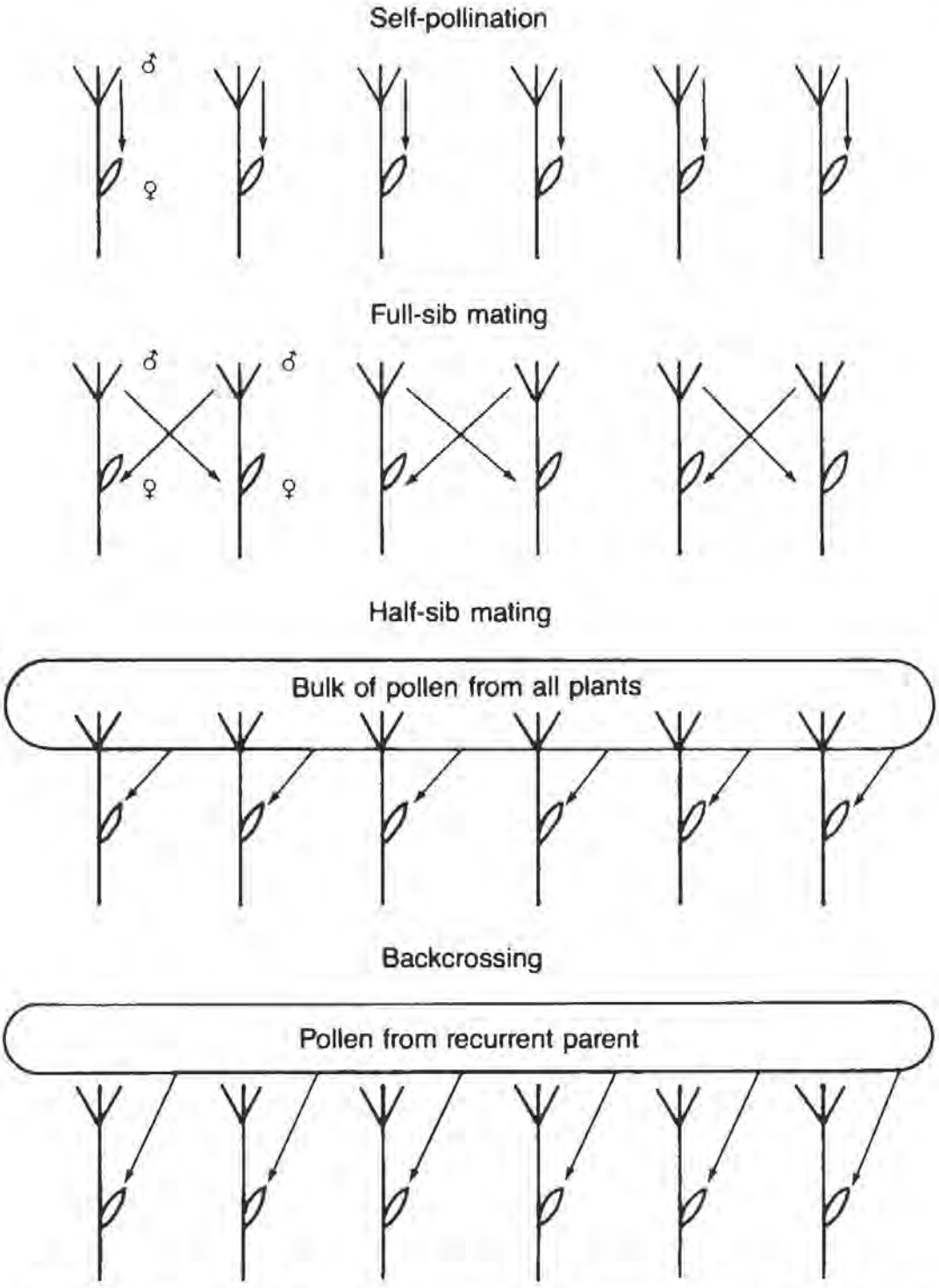


Figure 8-1 Four methods of inbreeding a population of plants with maize used as the example. The pollen of maize is produced in the tassel and the eggs are present in the ear. For each method of inbreeding, one or more seeds from each individual in a population is used collectively to plant the next generation.

Table 8-1 Calculation of Coefficients of Inbreeding for Five Methods of Inbreeding in a Diploid Species*

Generation of Inbreeding†	Self-Pollination	Full-Sib	Half-Sib	Backcrossing—Recurrent parent	
				Inbred ($F = 1$)	Noninbred ($F = 0$)
	$F = \frac{1}{2}(1 + F')$	$F = \frac{1}{4}(1 + 2F' + F'')$	$F = \frac{1}{8}(1 + 6F' + F'')$	$F = \frac{1}{2}(1 + F')$	$F = \frac{1}{4}(1 + 2F')$
1	$\frac{1}{2}(1 + 0) = \frac{1}{2}$	$\frac{1}{4}(1 + 2 \cdot 0 + 0) = \frac{1}{4}$	$\frac{1}{8}(1 + 6 \cdot 0 + 0) = \frac{1}{8}$	$\frac{1}{2}(1 + 0) = \frac{1}{2}$	$\frac{1}{4}(1 + 2 \cdot 0) = \frac{1}{4}$
2	$\frac{1}{2}(1 + \frac{1}{2}) = \frac{3}{4}$	$\frac{1}{4}(1 + 2 \cdot \frac{1}{4} + 0) = \frac{3}{8}$	$\frac{1}{8}(1 + 6 \cdot \frac{1}{8} + 0) = \frac{7}{32}$	$\frac{1}{2}(1 + \frac{1}{2}) = \frac{3}{4}$	$\frac{1}{4}(1 + 2 \cdot \frac{1}{4}) = \frac{3}{8}$
3	$\frac{1}{2}(1 + \frac{3}{4}) = \frac{7}{8}$	$\frac{1}{4}(1 + 2 \cdot \frac{3}{8} + \frac{1}{4}) = \frac{1}{2}$	$\frac{1}{8}(1 + 6 \cdot \frac{7}{32} + \frac{1}{8}) = \frac{39}{128}$	$\frac{1}{2}(1 + \frac{3}{4}) = \frac{7}{8}$	$\frac{1}{4}(1 + 2 \cdot \frac{3}{8} = \frac{7}{16}$
4	$\frac{1}{2}(1 + \frac{7}{8}) = \frac{15}{16}$	$\frac{1}{4}(1 + 2 \cdot \frac{1}{2} + \frac{3}{8}) = \frac{19}{32}$	$\frac{1}{8}(1 + 6 \cdot \frac{39}{128} + \frac{7}{32}) = \frac{195}{512}$	$\frac{1}{2}(1 + \frac{7}{8}) = \frac{15}{16}$	$\frac{1}{4}(1 + 2 \cdot \frac{7}{16}) = \frac{15}{32}$

*F = coefficient of inbreeding; F' = coefficient of previous generation; F'' = coefficient of second generation removed.

Source: Hallauer and Miranda, 1981.

†$F = 0$ for the F_2 generation or for noninbred plants of a random-mated population. Generation 1 is the first generation after the F_2.

Self-pollination and backcrossing to an inbred parent are the most extreme forms of inbreeding, followed by full-sib mating (Table 8-1). Homozygosity will be achieved eventually under all systems of inbreeding except backcrossing to a noninbred parent. With a noninbred parent, the inbreeding coefficient can reach a maximum of 0.5.

Variation in the approach to homozygosity among the self, full-sib, half-sib, and backcross systems of mating is associated with differences in the opportunity for identical alleles to come together. A slower fixation of undesirable alleles allows more opportunities for selection during inbreeding. In the choice of a system of inbreeding, the importance of selection must be weighed against the length of time required to attain the desired level of homozygosity.

INBREEDING DEPRESSION IN AN AUTOPOLYPLOID SPECIES

The coefficient of inbreeding for an autotetraploid species undergoing self-pollination was described by Kempthorne (1957) as

$$F = \tfrac{1}{6}\,[1 + 2\alpha + (5 - 2\alpha)\,F']$$

where F = probability that two alleles at a locus are identical by descent

α = probability of double reduction, which results in gametes that have alleles from sister chromatids

F' = coefficient of inbreeding for preceding generation

When a random-mated population is inbred one generation by self-pollination, the coefficient of inbreeding becomes $\frac{1}{6}$ if $\alpha = 0$. F' equals 0 because the coefficient of inbreeding in a random-mated population is zero.

$$F = \tfrac{1}{6}\,\{1 + 2(0) + [5 - 2(0)]\,0\} = \tfrac{1}{6}$$

The change in the coefficient of inbreeding by self-pollination in an autotetraploid is illustrated in Fig. 8-2. Four identical alleles at a locus are required to achieve homozygosity in an autotetraploid compared with only two in a diploid; therefore, homozygosity is achieved less rapidly in an autotetraploid.

Inbreeding depression in autopolyploids has been found to exceed that predicted by the coefficient of inbreeding. In alfalfa, an autotetraploid species, the decrease in forage yield with one generation of self-pollination has been twice as large as that predicted by the percentage increase in homozygosity (Aycock and Wilsie, 1968) (Fig. 8-3). This response to inbreeding has been attributed to a decrease in favorable interactions among multiple alleles at a locus.

The change in percentage of homozygosity in autotetraploids as measured by the coefficient of inbreeding does not take into account changes in the number of loci with two, three, or four different alleles (Chap. 4). The number of different alleles at a locus is considered a factor in the expression of inbreeding depression by autopolyploids. Busbice and Wilsie (1966) described in detail the theoretical

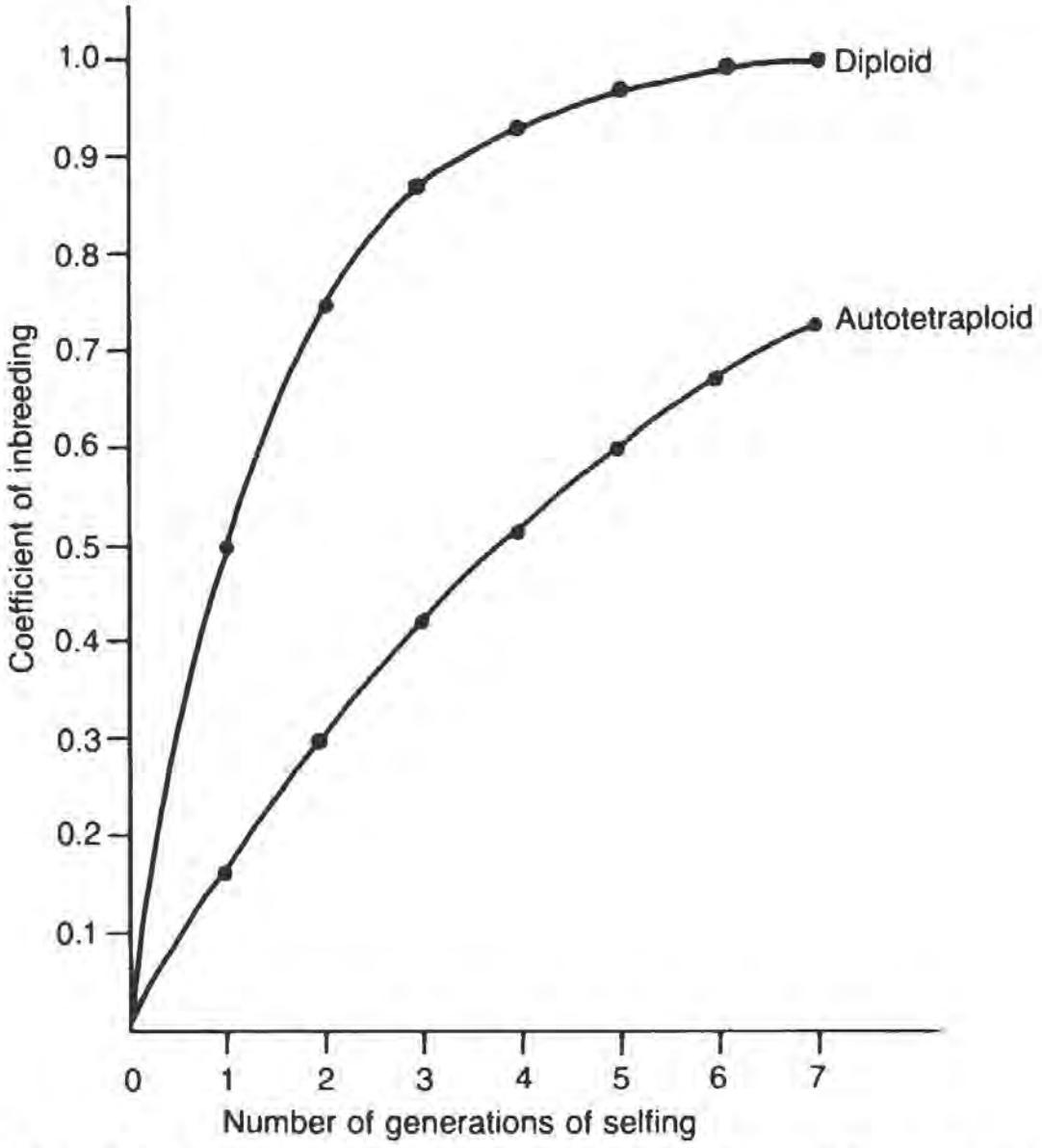


Figure 8-2 Relationship of the coefficient of inbreeding and the number of generations of self-pollination in a diploid and an autotetraploid species.

basis for considering intralocus interactions in predicting the change in performance of autotetraploid alfalfa associated with inbreeding. They defined the genotypic values (G) for loci with one, two, three, or four alleles ($abcd$) by the additive value of alleles and allelic interactions. First-order interactions involve two alleles (ab), second-order interactions involve three alleles (abc), and third-order interactions involve all four alleles.

$$G_{abcd} = a + b + c + d + (ab) + (ac) + (ad) + (bc) + (bd) + (cd) + (abc) + (abd) + (acd) + (bcd) + (abcd)$$

$$G_{aabc} = a + a + b + c + (ab) + (ac) + (bc) + (abc)$$

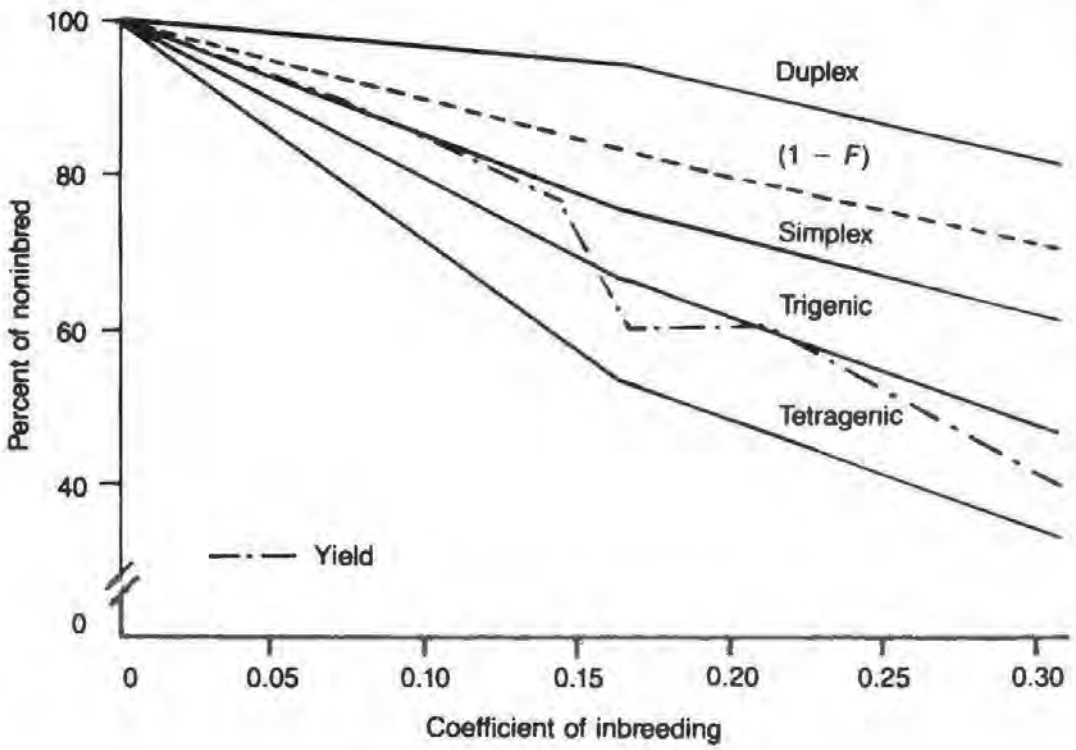


Figure 8-3 Comparison of the yield of alfalfa for various levels of inbreeding and the theoretical loss of first-order interactions from a tetragenic, trigenic, duplex, and simplex locus. (Courtesy of Aycock and Wilsie, 1968.)

$$G_{aabb} = a + a + b + b + (ab)$$
$$G_{aaab} = a + a + a + b + (ab)$$
$$G_{aaaa} = a + a + a + a$$

If intralocus interactions contribute to the performance of an individual, a reduction in the frequency of tetragenic and trigenic loci could cause inbreeding depression that would not be accounted for by the increase in the frequency of homozygous loci. Self-pollination of a tetragenic locus would produce one-sixth tetragenic, two-thirds trigenic, and one-sixth duplex loci. This would decrease substantially the frequency of intralocus interactions with no increase in frequency of homozygous loci.

Because of their multiallelism, autopolyploid species can accumulate a greater number of masked deleterious recessive alleles than diploid species. The uncovering of accumulated deleterious alleles during selfing may contribute to the greater degree of inbreeding depression expressed in autopolyploids than is predicted by the coefficient of inbreeding.

INBREEDING IN SMALL POPULATIONS

The need for progeny evaluation and recombination in recurrent selection programs often necessitates the use of population sizes of 200 individuals or less.

Closed populations are commonly utilized so that the effects of a specific selection method can be determined.

The amount of heterozygosity lost each generation in a random-mated population can be influenced by the population size. In populations of monoecious diploid plants undergoing random mating, including self-pollination, heterozygosity decreases by approximately $\frac{1}{2}N$ each generation, where N is the number of unrelated individuals in the population that are mated. In populations in which self-pollination is prevented, the decrease in heterozygosity each generation is approximated by $1/(2N + 1)$.

Effective population size, N_e, is a relative measure of the number of parents used to form a breeding population. It does not represent the number of individuals from a population that are tested in a recurrent selection program. Effective population size is dependent on the level of inbreeding of the parents that are mated and the number of gametes contributed to the next generation by each sex (Hallauer and Miranda, 1981). The general equation for calculating the effective population size is

$$N_e = \frac{4N_mN_f/(N_m + N_f)}{1 + F_p}$$

where N_m = number of male parents

N_f = number of female parents

F_p = coefficient of inbreeding of parents

Table 8-2 Expected Levels of Inbreeding with Varying Effective Population Sizes for Populations Undergoing Recurrent Selection by Evaluation and Recombination of $S_{0:1}$ lines ($F_p = 0$) or $S_{1:2}$ lines ($F_p = 0.5$)

Cycle of Selection	Inbreeding of Population (F)					
	20 Parents		30 Parents		40 Parents	
	$S_{0:1}$(20*)	$S_{1:2}$(13.3)	$S_{0:1}$(30)	$S_{1:2}$(20)	$S_{0:1}$(40)	$S_{1:2}$(26.7)
1	0.05	0.08	0.03	0.05	0.02	0.04
2	.10	.14	.06	.10	.05	.07
3	.14	.20	.09	.14	.07	.10
4	.18	.25	.12	.18	.10	.14
5	.22	.31	.15	.22	.12	.17
6	.25	.35	.18	.26	.14	.20
7	.29	.40	.20	.29	.16	.23
8	.32	.44	.23	.32	.18	.26
9	.35	.48	.26	.36	.20	.28
10	.39	.52	.28	.39	.22	.31
20	.62	.76	.48	.62	.39	.52
40	0.86	0.94	0.73	0.86	0.63	0.77

*Effective population size (N_e). $N_e = 2N/(1 + F_p)$, where N is the number of parents recombined and F_p is the coefficient of inbreeding of the parents recombined.

Source: Hallauer and Miranda (1981).

When equal numbers of males and females contribute the same number of gametes to the next generation, such as occurs when selfed lines are used for mating, the equation becomes

$$N_e = \frac{2N}{1 + F_p}$$

where N is the number of individuals that are mated.

The implications of effective population size on population improvement by recurrent selection have been analyzed by several authors, as summarized by Hallauer and Miranda (1981). In brief, inbreeding in a population increases as the effective population size decreases (Table 8-2). As inbreeding increases, genetic variability among individuals within the population is reduced, which results in lower genetic gain from recurrent selection (Chap. 17).

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CHAPTER NINE

Heterosis

Hybrid cultivars are used for the commercial production of a number of plant species. They are a desirable type of cultivar because of their ability to capitalize on heterosis. Heterosis is the superiority in performance of hybrid individuals compared with their parents. The occurrence of heterosis is common in plant species, but its level of expression is highly variable.

MEASUREMENT OF HETEROSIS

The performance of a hybrid relative to its parents can be expressed in two ways. Mid-parent heterosis is the performance of a hybrid compared with the average performance of its parents. High-parent heterosis is a comparison of the performance of the hybrid with that of the best parent in the cross.

Heterosis usually is expressed as a percentage and computed as follows:

$$\text{Mid-parent heterosis (\%)} = \frac{F_1 - \text{MP}}{\text{MP}} \times 100$$

$$\text{High-parent heterosis (\%)} = \frac{(F_1 - \text{HP})}{\text{HP}} \times 100$$

where F_1 = performance of hybrid

MP = average performance of parents per se (parent 1 + parent 2)/2

HP = performance of best parent

For example, assume that the yield of a hybrid is 90 units, that of parent 1 is 60 units, and that of parent 2 is 80 units. The average performance of the parents would be 70 units.

$$\text{Mid-parent heterosis} = \frac{90 - 70}{70} \times 100 = 28.6\%$$

$$\text{High-parent heterosis} = \frac{90 - 80}{80} \times 100 = 12.5\%$$

GENETIC BASIS OF HETEROSIS

Heterosis can be expressed when the parents of a hybrid have different alleles at a locus and there is some level of dominance among those alleles (Falconer, 1981). There has been extensive debate concerning the relationship between level of dominance and expression of heterosis. The two hypotheses that have received the most attention are the dominance hypothesis and the overdominance hypothesis. According to the dominance hypothesis, heterosis is caused by complete or partial dominance. In the overdominance hypothesis, the value of the heterozygote is considered superior to the value of either homozygote.

The difference between the two hypotheses can be illustrated with the cross *AAbbCC* × *AABBcc*. Assume that the amount of performance contributed by the *A* allele is 10 units, that of *B* is 12, that of *b* is 6, that of *C* is 8, and that of *c* is 4. By substituting these values for each allele, the average of the alleles *AA* would be 10, of *BB* would be 12, of *bb* would be 6, of *CC* would be 8, and of *cc* would be 4. The performance of the *AAbbCC* parent would be $10 + 6 + 8 = 24$, and that of *AABBcc* would be $10 + 12 + 4 = 26$. The performance of the hybrid will be unaffected by the *A* allele because this allele contributes equally to the expression of both parents and the hybrid. If there is no dominance, the value for the *Bb* and *Cc* loci will be the average of the two alleles, $Bb = (12 + 6)/2 = 9$ and $Cc = (8 + 4)/2 = 6$, and no heterosis will be expressed in the hybrid: $AABbCc = 10 + 9 + 6 = 25$. If partial or complete dominance is present, as is assumed by the dominance hypothesis, the value of the heterozygote will be greater than the average of the two alleles at a locus. For example, assume that there is partial dominance for *Bb*, which gives it a value of 10, and for *Cc*, which has a value of 7. The hybrid would express both mid-parent and high-parent heterosis: $AABbCc = 10 + 10 + 7 = 27$. The maximum values for the heterozygous loci *Bb* and *Cc* under the dominance hypothesis are those achieved with complete dominance, $BB = Bb = 12$ and $CC = Cc = 8$. In this case, the hybrid would express even greater heterosis than with partial dominance: $AABbCc = 10 + 12 + 8 = 30$.

For the overdominance hypothesis, the value of the heterozygote exceeds that which is possible with complete dominance. The value of *Bb* would be greater than that of *BB* or *bb* ($Bb > 12$), and the value of *Cc* would be greater than that of *CC* or *cc* ($Cc > 8$). If $Bb = 13$ and $Cc = 9$, the *AABbCc* hybrid would have a value of $10 + 13 + 9 = 32$.

There are arguments for and against both of the hypotheses. The dominance

hypothesis was proposed by Bruce (1910) and received strong support from Jones and others (Jones 1917,1945,1958). It is considered a reasonable explanation of heterosis because it is based on levels of dominance that have been widely observed for qualitative characters. In contrast, evidence for the expression of overdominance for qualitative characters is limited.

Several arguments against the dominance hypothesis have been presented (Hallauer and Miranda, 1981). If complete dominance is the maximum expression of a heterozygote, it should be possible to develop a homozygous segregate from a cross that is equal in performance to the hybrid. For example, the cross of *AAbbCC* × *AABBcc* should produce F_2 progeny with the genotype *AABBCC* that are equal in performance to the hybrid *AABbCc*. In crops such as maize, inbred lines have not been identified that are equal in yield to the best hybrid. This inability to obtain inbreds that are equal in performance to hybrids is cited as evidence against the dominance hypothesis. In rebuttal of this argument, it has been pointed out that the probability of recovering all favorable dominant alleles in one homozygous individual is limited when parents differ for a large number of alleles controlling a quantitative character (Collins, 1921).

If complete or partial dominance is present, the frequency distribution of F_2 progeny from a cross should be skewed toward the dominant phenotype. With overdominance, a normal distribution would be expected. The F_2 distributions for yield and other quantitative characters generally are normal, a fact that is used as evidence against the dominance hypothesis. As a refutation of this criticism of the dominance hypothesis, it has been indicated that the amount of skewness expected is limited when the number of loci controlling a character is large (Collins, 1921).

The overdominance hypothesis proposed by Shull (1908) was supported by East (1936), Hull (1945), and others. The primary argument against the overdominance hypothesis is the extensive amount of data that demonstrate the importance of partial or complete dominance for quantitative characters and the limited amount of similar evidence for the importance of overdominance. Hallauer and Miranda (1981) reviewed the existing data for quantitative characters of maize and concluded that the evidence supports the dominance hypothesis as the genetic basis of heterosis.

The possible role of epistasis in the expression of heterosis also has been considered. Epistasis involves the interaction of alleles at two or more loci that could result in performance superior to that of independent loci. There is evidence for the presence of epistasis in the expression of quantitative characters, but epistasis seems to be considerably less important than dominance (Hallauer and Miranda, 1981).

HETEROSIS IN DIPLOID CULTIVARS

In a diploid species with two alleles at a locus, the average heterosis of a cross is greatest for a single-cross hybrid due to the occurrence of the greatest possible

number of loci with a dominant allele. For example, the mating of the inbreds *AABBccdd* × *aabbCCDD* results in single-cross individuals with a dominant allele at each locus, *AaBbCcDd*. Dominant alleles at each of the four loci for all single-cross individuals provide the highest average performance for the cross.

A three-way hybrid is produced by the mating of a single-cross hybrid to a third inbred parent. The average heterosis expressed by the three-way hybrid depends on the frequency of loci that retain a dominant allele. This is a function of the genetic relationship between the genotype of the single cross and the third parent. The frequency of loci with a dominant allele generally will be less in the three-way than in the single cross. Consider the mating of the single cross *AaBbCcDd* with the inbred *AABBccdd*. The genotypes of the hybrid progeny are *A__B__CcDd*, *A__B__Ccdd*, *A__B__ccDd*, *A__B__ccdd*. Because of the occurrence of homozygous recessive loci in some of the progeny, the frequency of loci with dominant alleles and the average heterosis in the three-way cross is less than in the single cross.

In a double-cross hybrid formed by mating two single crosses, the average frequency of loci with a dominant allele and average heterosis would be less than in a three-way hybrid. Consider the mating of two single crosses with identical genotypes, *AaBbCcDd* × *AaBbCcDd*. Homozygous recessive alleles at one or more loci in some of the progeny of this mating would reduce the average heterosis of the cross.

HETEROSIS IN HYBRID CULTIVARS OF AUTOPOLYPLOID SPECIES

The potential use of hybrid cultivars for autopolyploid species has been evaluated, particularly for alfalfa. The expression of heterosis has been associated with the interaction of different alleles at a locus.

The number of different alleles at a locus in an autotetraploid can range from one to four. It has been proposed that maximum heterosis is expressed by a tetragenic locus (*abcd*), and declines for a trigenic (*abcc*), digenic (*aaab*), and monogenic locus (*aaaa*) (Busbice and Wilsie, 1966). Under this assumption, any mating system that enhances the frequency of loci with multiple alleles would maximize the expression of heterosis. Dunbier and Bingham (1975) tested this hypothesis by deriving populations with equivalent gene frequencies but different levels of intralocus heterozygosity. The populations with the highest expected frequency of tetragenic and trigenic loci were found to have the greatest forage yield, seed weight, and fertility.

The frequency of loci with multiple alleles in a hybrid is associated with the level of inbreeding of the parents. Double-cross hybrids have more multiallelic loci than do single-cross hybrids when parents are inbred. Consider the extreme example in which there are four homozygous autotetraploid parents, each with a different allele at a locus: *aaaa*, *bbbb*, *cccc*, and *dddd*. Single crosses between any two parents would result only in digenic loci, such as *aaaa* × *bbbb* → *aabb*.

A double cross between the four parents would result in a tetragenic locus, *abcd*. In contrast, single-cross hybrids have more multiallelic loci on the average than do double crosses when the parents are noninbred.

IMPLICATIONS OF HETEROSIS ON CULTIVAR DEVELOPMENT

The possibility of utilizing hybrid cultivars for commercial production has been examined for virtually every crop species. The two primary motivations for the interest are increased productivity of the crop and the desire to have a seed product that must be purchased each year by the farmer.

Heterosis has been observed for seed or forage yield in essentially all crop species, but the level of heterosis is widely different among species. In general, heterosis is greatest in cross-pollinated crops and least in self-pollinated species. The commercial use of hybrids is restricted to those crops in which the amount of heterosis is sufficient to justify the extra cost required to produce hybrid seed (Chap. 35).

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CHAPTER TEN

Parent Selection

One of the most important decisions a plant breeder must make involves the selection of parents for population development. The decision-making process includes identifying the characters to be improved, understanding how the characters are inherited, and identifying sources of parental germplasm.

CHARACTERS TO BE IMPROVED

The proper selection of parental germplasm begins with a clear understanding of the goal of the breeding project. Breeding a better cultivar is not an adequate statement of the objective, because the breeder can consider many characters for improvement. The methods used in a cultivar development program and their probability of success are dependent on the number of characters to be improved simultaneously. The proportion of desired individuals for multiple characters in a population is obtained by multiplying together the proportion of desired individuals expected in the population for each character to be improved. This assumes that the characters are inherited independently, i.e., are not genetically correlated. As an illustration of the principle, assume that there are five characters, A through E, that could be improved in developing cultivars of a species. The proportion of desired individuals in a population for character A is $^1/_4$, B $^1/_8$, C $^1/_{20}$, D $^1/_{60}$, and E $^1/_{100}$. If improvement of all five characters were the objective, the proportion of desired individuals would be $^1/_4 \times {}^1/_8 \times {}^1/_{20} \times {}^1/_{60} \times {}^1/_{100} = {}^1/_{3,840,000}$. Selection for characters A, B, C, and D would involve the proportions $^1/_4 \times {}^1/_8 \times {}^1/_{20} \times {}^1/_{60} = {}^1/_{38,400}$, and selection for characters A, B, and C would involve $^1/_4 \times {}^1/_8 \times {}^1/_{20} = {}^1/_{640}$. The probability of success would be greater by considering improvement of characters A, B, and C, instead of A through D or A through E.

The breeder generally is required to assign priorities to the characters that

could be considered for improvement. Most breeding programs have one or a few characters that are of paramount importance. In most instances, these are the characters that are of greatest economic importance. They would include plant or seed yield, for many agronomic crops; attractiveness of the flower or plant, for ornamental species; and flavor and color, for fruit crops. The characters of major importance are given first priority in determining the parents that will be used for population development.

In determining the characters of highest priority, it is important for the breeder to clearly understand the requirements of consumers, be they farmers, manufacturers, or the end users. It is not uncommon to hear a breeder say that the consumer demands a certain characteristic. Such a statement must be based on fact, not personal opinion. For example, lodging resistance is a trait considered important by many breeders of agronomic crops. One of the most widely grown soybean cultivars in the midwestern United States during recent years was 'Corsoy,' a lodging-susceptible cultivar. Farmers commonly said that the only good characteristic of the cultivar was its high yield. The widespread use of 'Corsoy' indicated that farmers considered yield a first priority and lodging resistance of secondary importance. The breeder can limit the characters considered in a breeding program by paying attention to the real demands of the consumer. A direct means of acquiring information on consumer preference is an appropriate survey. Reinhart (1979) surveyed randomly selected oat growers in Iowa to identify production practices used by farmers, as well as the characteristics the growers considered important when selecting a cultivar for commercial production. High grain yield was a clearly preferred characteristic, as were medium plant height and medium time of maturity.

INHERITANCE OF THE CHARACTER TO BE IMPROVED

The inheritance of characters ranges from control by one major gene whose expression is not influenced by the environment (qualitative characters) to control by many genes and much influence by the environment (quantitative characters). Selection of appropriate parents for a qualitative character controlled by a single major gene is relatively easy, because the breeder can determine through appropriate tests if the parent has the gene or does not. At least one parent of a cross must have the gene for it to be recovered in the progeny. Specific resistance to a plant pest often is controlled by a single major gene. The breeder chooses one or more parents that possess the necessary gene when developing a population for selection of cultivars that must have resistance. The same principle applies for characters controlled by one or a few major genes or quantitative characters with a high heritability. Time of maturity is a quantitative character in most species. The distribution for maturity among progeny in a population will be closely associated with maturity in the parents selected. It is possible to obtain transgressive segregation for maturity within a population, but the frequency of

such segregates would be less than the frequency of offspring with a maturity within the range of that of the parents.

Selection of parents is more difficult for characters that are to be improved beyond the level that is present in available germplasm. Yield is an example of a quantitative character that breeders attempt to improve beyond the level of that present in current cultivars. The selection of parents for such characters generally involves selection of elite germplasm with the best performance for the character and the greatest genetic diversity available. The common practice of crossing together elite lines, referred to as making good × good crosses, has narrowed the genetic diversity among commercial cultivars of most species (National Academy of Science, 1972). This decreases the array of alleles available among parents for continued improvement of the species. Unfortunately, the most elite breeding lines often have the least amount of genetic diversity, because they were selected as the progeny of parents with similar ancestry. Plant breeders attempt to develop populations from elite parents that have the most diverse ancestry possible, to increase the chance of obtaining a superior progeny with different favorable alleles from all of the parents.

SOURCES OF PARENTAL GERMPLASM

The breeders of most species have several sources of parental germplasm available to them. The soures can be subdivided into classes on the basis of their similarity to commercially grown cultivars of the crop.

Commercial Cultivars

The parents most widely used by plant breeders are current cultivars that are grown commercially, the inbred parents of hybrid cultivars, or parents of synthetic cultivars. These cultivars represent the most elite source of germplasm for characters of major importance. The mean performance of a population developed from commercial cultivars will be high, and the probability of obtaining superior progeny with no major weaknesses is good. On the other hand, if the genotypes of the cultivars used for population development are similar, the possibility of obtaining adequate genetic variability in the population to achieve major improvement in a character may be small.

Elite Breeding Lines

Lines in advanced stages of testing are a useful source of parental germplasm. The lines may be destined for release as cultivars or may closely approach that

level of performance. Plant breeders increase their potential genetic gain per year by using breeding lines as parents as soon as the superiority of the lines is identified (Chap. 17). The availability of elite breeding lines generally is limited to the originator. Public breeders commonly exchange elite breeding lines with each other, as do private breeders within the same company. Exchange of elite breeding lines among public institutions and private companies is determined by the policy of the institutions involved. In the United States, public universities and the U.S. Department of Agriculture have guidelines for release of germplasm to the public, referred to as the ESCOP policy (App. B). The ESCOP policy provides the guidelines for germplasm release from public institutions, but each institution has its own system for determining what qualifies for release and how the germplasm will be distributed. The release of breeding lines from private companies generally involves a specific request from one breeder to another.

Acceptable Breeding Lines with Superiority in One or a Few Characters

Breeders commonly have breeding lines that do not have the overall performance required for a cultivar but are superior for one or a few characters. These lines would include old cultivars that have been replaced by newer cultivars with better overall performance. Despite the lack of overall performance, the older cultivars may have a few characters in which they excel. Plant breeders employed by public universities and the U.S. Department of Agriculture frequently release lines that have been improved for one or a few characters to a level unavailable in commercial cultivars and elite breeding lines. These improved lines are referred to as genetic stocks or germplasm lines. For example, Iowa State University released a germplasm line of soybean designated A2 (Fehr and Bahrenfus, 1980). It did not have adequate yield potential to be released as a cultivar, but possessed useful resistance to iron-deficiency chlorosis on calcareous soil.

Plant Introductions of the Cultivated Species

Plant introductions and collections of species native to the United States are maintained as a source of parental germplasm (Chap. 11). They often have several characters that are unacceptable commercially, but possess a specific character of value. Genes for pest resistance commonly are obtained from this source of germplasm. For example, although the soybean plant introduction 'Peking' has black seed and is unacceptable for commercial production in the United States, it has been used as an important source of resistance to the soybean cyst nematode.

Related Species

When a desirable characteristic is lacking in the cultivated species, breeders consider related species as a source of parental germplasm. The related species generally are unacceptable for many characteristics and may not readily hybridize with the cultivated species. Nevertheless, they may possess characteristics of significant value, particularly with respect to pest resistance. Breeders and geneticists have developed techniques for interspecific hybridization that facilitate the transfer of characters between species (Chap. 14).

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CHAPTER ELEVEN

Plant Introduction and Genetic Diversity

The movement of seeds and plants from one area of the world to another has been an important factor in agricultural development. The crops currently produced in a country often originated in other parts of the world. Plant introduction continues to be important in the identification of new crops and in obtaining new parental material for the development of cultivars of existing crops. This chapter will consider the overall concept of plant introduction; the procedures for securing, maintaining, and distributing plant introductions; and the techniques necessary to utilize them effectively for genetic improvement of a species.

ORIGIN OF GENETIC VARIATION IN NATURE

The center of origin for a crop is the geographical area in which it originated. A center of diversity is a location where there is extensive genetic variability among genotypes of the cultivated species and of related species. The center of origin and center of diversity for a crop may be the same or may be different.

The person credited with formulating and implementing the first large-scale program for germplasm collection is N. I. Vavilov. He was interested in obtaining germplasm with a broad range of genetic variability from throughout the world to use in improving cultivated crops in Russia. His work marked the beginning of a systematic approach to plant germplasm collection, maintenance, and utilization that has been vital to the improvement of crop species. On the basis of collections made by Russian scientists, Vavilov (1926, 1951) proposed eight centers of origin, two of which were later designated as subcenters (Table 11-1).

Detailed research on the origin of cultivated plants since the time of Vavilov has demonstrated that a center of origin cannot be defined simply by the genetic

Table 11–1 Centers of Origin of Plant Species, According to N.I. Vavilov (Vavilov, 1951)

Centers of Origin		Examples of Cultivated Plants
I.	Chinese	Lettuce, rhubarb, soybean, turnip
II.	Indian	Cucumber, mango, oriental cotton, rice
IIa.	Indo-Malayan	Banana, coconut, yam
III.	Central Asiatic	Almond, cantaloupe, flax, lentil
IV.	Near-Eastern	Alfalfa, apple, cabbage, rye
V.	Mediterranean	Celery, chick pea, durum wheat, peppermint
VI.	Ethiopian (formerly Abyssinian)	Castor, coffee, grain sorghum, pearl millet
VII.	South Mexican and Central American	Lima bean, maize, papaya, upland cotton
VIII.	South American (Peruvian-Ecuadorian-Bolivian)	Egyptian cotton, potato, pumpkin, tomato
VIIIa.	Chiloé	Potato
VIIIb.	Brazilian-Paraguayan	Manioc, peanut, pineapple, rubber tree

diversity of a crop present in an area. Vavilov's original concept of eight centers of origin has been revised several times. The complexity of determining where a crop originated was described by J. R. Harlan (1971). He indicated that an assessment of the origin and dispersal of cultivated plants requires information from many fields of investigation, including genetics, chemotaxonomy, numer-

Table 11-2 Centers and Noncenters of Origin of Some Cultivated Plants, as Proposed by Harlan (1975)

Center		Noncenter	
A1:	Near East	A2:	Africa
	oat		sorghum
	cabbage		okra
	smooth bromegrass		sudangrass
	flax		oil palm
	cherry		watermelon
B1:	China	B2:	Southeast Asia and Pacific Islands
	rice		jackbean
	cucumber		eggplant
	soybean		coconut
	rapeseed		sugarcane
	peach		banana
C1:	Mesoamerica	C2:	South America
	maize		peanut
	squash		peanut
	upland cotton		tobacco
	sunflower		lima bean
	pineapple		pepper

ical taxonomy, ecology, geography, archeobotany, paleobotany, religion, history, art, and geology. Harlan suggested that the domestication of crop plants may have occurred in both centers and noncenters. In this case, a center is a limited geographical area where a crop was domesticated and from which it was disbursed to other regions of the world; a noncenter is a broad geographical area in which a crop may have been domesticated simultaneously in several different locations. Harlan proposed three centers and three noncenters. The centers are the Near East, China, and Mesoamerica and the noncenters are Africa, Southeast Asia and Pacific Islands, and South America (Table 11-2).

ACQUISITION OF PLANT INTRODUCTIONS

The procedures that have been used to obtain plant introductions for the United States are the same in principle as those that would be used in any country.

Germplasm Collection

The collection of plants in foreign countries has been an important method of obtaining plant introductions. Persons who make planned collections of plants are referred to as plant explorers or genetic resource collectors. They collect diverse genotypes of cultivated, wild, and weedy species that may have potential as breeding material in the future.

Collections of a species or its relatives are made in areas in which a high level of genetic diversity is present. They also are made in areas with specialized environments in which unique genotypes may have evolved through natural selection.

A major concern today is the rapid loss of genetic diversity in crop species throughout the world. Extensive genetic diversity for a cultivated species occurs where a crop has been grown for a long time and where cultivars developed by plant breeders are not widely used. In such areas, the seeds or vegetative propagules planted by farmers may be a random sample of the previous crop that was subjected to natural selection. The farmer might perform some artificial selection by using seeds or propagules from a group of individuals with preferred characteristics. When a crop is heterogeneous and contains many different genotypes, natural and artificial selection result in the development of thousands of different cultivars, commonly referred to as landraces. If 100 farmers use seed from their own crop year after year without exchanging seed with each other, 100 different landraces can develop, each with a unique genetic constitution. Landraces are a valuable source of genetic diversity for improvement of a cultivated species. They are rapidly being lost as farmers are provided with improved cultivars developed by plant breeders.

It is important that landraces be collected and preserved throughout the world, particularly in areas where modern cultivars are replacing germplasm that has

been used for crop production for a long time. Harlan (1975) found that widespread acceptance of semidwarf Mexican spring wheat cultivars in Asia, the center of diversity for the species, has caused the rapid elimination of many landraces of the crop in some regions. Political instability and crop failures also can eliminate valuable genetic resources.

Genetic diversity present in wild and weedy species is lost as the amount of land that remains undisturbed by people decreases and as the intensity of crop production increases. By definition, a wild species is one that grows in undisturbed areas. Genotypes of wild species are destroyed as previously undisturbed land is utilized for crop production, grazing, or industrial and domestic purposes. Certain types of environmental pollution also have caused the loss of germplasm of wild species.

Weedy species are uncultivated species that grow in areas that have been disturbed by agricultural production. The increased emphasis on weed control in cultivated crops has eliminated genotypes of species that previously were tolerated in agricultural production.

The collection of germplasm is difficult because it is not possible to sample every individual in a heterogeneous population of plants. The objective is to obtain a high level of genetic diversity with a manageable number and size of samples (Hawkes, 1981). The collector must determine if plants in a population will be sampled at random or if those with particular characteristics will be chosen. An appropriate distance between collection sites must be determined on the basis of variation in the environment to which plants are exposed, including such factors as cropping practices, soil, temperature, and moisture. For each sample, the collector records field information that will be helpful in preserving, evaluating, and utilizing the germplasm.

Exchange of Germplasm

The exchange of germplasm between countries is an important source of plant introductions. The genotypes contained in the germplasm collections of various countries may not be the same because of differences in the places from which samples have been obtained. The exchange of germplasm may involve entire collections of a species or only particular genotypes with special characteristics. One example of the benefits to be derived from international germplasm exchange is the development of semidwarf wheat cultivars in North America. Two dwarfing genes were found in a short, stiff-strawed Japanese wheat cultivar, which was crossed in Japan to the improved American cultivars 'Fultz' and 'Turkey.' A dwarf cultivar, 'Norin 10,' was released in 1935 to Japanese farmers. 'Norin 10' was brought to the United States in 1946. The use of 'Norin 10' in the wheat breeding program at Washington State University led to the development of the semidwarf wheat cultivar 'Gaines,' which was widely grown in the Pacific Northwest of the United States. A 'Norin 10' derivative from the Washington

State program was supplied to breeders in Mexico, who crossed it to indigenous Mexican cultivars. Several short-statured spring wheat cultivars selected from the crosses were first grown by Mexican farmers in 1962. Mexican wheat yields, which had leveled off in the late 1950s, began to increase with the introduction of the semidwarf cultivars. The same cultivars were introduced to India, Pakistan, the United States, and other countries. It is estimated that the dwarfing genes from 'Norin 10' have affected the food supply of one-quarter of the people of the world.

International germplasm exchange also can be valuable when a new pathogen or pest becomes a problem. Maize production declined in West Africa following the accidental introduction of maize rust (*Puccinia polysora*) in 1949. Beginning in 1952, when the rust was first observed in East Africa, plant breeders in Kenya screened 68 East African lines and found no resistance. Examination of herbarium specimens showed that the origin of the rust was Central and South America. Lines of maize from Central America and the Caribbean were brought to Kenya and 45 of the 203 lines screened were resistant. By backcrossing genes from the resistant lines into adapted African lines, maize lines with adequate rust resistance were available by 1956.

Gifts of Germplasm

Plant germplasm has been used as political, scientific, and personal gifts between people from different countries. Germplasm that is obtained as a gift can be a useful source of plant introductions, if it is shared with the public agency responsible for germplasm conservation and distribution.

Purchase of Germplasm

The purchase of germplasm from another country generally applies to improved cultivars that may be of direct commercial value. The developer of a cultivar in one country may charge a fee to permit its use in another country.

MAINTENANCE AND DISTRIBUTION OF GERMPLASM

The international plant germplasm network includes the U.S. National Plant Germplasm System (NPGS), as well as the plant germplasm systems of other countries. The International Board of Plant Genetic Resources (IBPGR) coordinates the activity of all components of the international system. IBPGR is a center in the Consultative Group on International Agriculture Research (CGIAR), which is part of the United Nations Food and Agriculture Organization (FAO).

Besides national systems of germplasm collection and preservation, the CGIAR research centers, such as the International Center for Maize and Wheat Improve-

ment (CIMMYT), the International Rice Research Institute (IRRI), and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) maintain extensive germplasm collections. The germplasm is available to scientists throughout the world.

Any individual in the United States can introduce seeds or propagules from another country if they comply with the requirements of the Animal and Plant Health Inspection Service of the U.S. Government. They have the choice of keeping the seeds for personal use or making them available to the public through the U.S. Plant Introduction system. Every individual who provides germplasm to the U.S. Plant Introduction system is contributing to present and future needs for genetic diversity.

Plant introductions are cataloged and assigned a plant inventory (PI) number by the Plant Introduction Office of the Plant Genetics and Germplasm Institute at Beltsville, Maryland (App. A). Introductions are then sent to the plant introduction station responsible for maintenance of the species. Most species are maintained at one of four Regional Plant Introduction Stations; however, some species are maintained at other sites. The environmental requirements a plant introduction has for seed production are the primary consideration in determining which station will be responsible for the plant's maintenance. Seed of plant introductions is maintained in long-term seed storage at the National Seed Storage Laboratory in Fort Collins, Colorado. Vegetatively propagated plant introductions are maintained at one of several National Clonal Repositories.

The NPGS maintains more than 400,000 accessions, both seeds and vegetatively propagated stocks. These are mostly landraces and unimproved germplasm from outside the United States. New accessions are added at a rate of 7000 to 15,000 per year.

Any individual in the United States can request and obtain seed of a plant introduction. The request can be made directly to the location that maintains the plant introduction or to one of the four regional stations. Requests are not sent to the National Seed Storage Laboratory because its seed supply is not available for routine distribution.

In 1984, the Germplasm Resources Information Network (GRIN) began operation. GRIN is a computerized data base containing information on the location, characteristics, and availability of accessions within the plant introduction system. Anyone with a valid need for information or for particular germplasm may obtain access to the system through the Database Management Unit of the Agricultural Research Service, Plant Genetics and Germplasm Institute, Beltsville, Maryland.

EVALUATION OF PLANT INTRODUCTIONS

Some characteristics of plant introductions are evaluated by the staff at the location where the plants are maintained, but the majority of the evaluation is

done by other persons. Evaluation generally is the first step in the utilization of plant introductions. An individual interested in a particular characteristic from plant introductions often will evaluate part or all of the collection of a species. It may be a plant pathologist searching for disease resistance or a physiologist interested in variability for photosynthetic efficiency. Every individual who receives a plant introduction is requested to return information on the characteristics that were evaluated. The information becomes a part of the record of the plant introduction that can be shared with other interested persons. In such a manner, each person who uses plant introductions contributes to their evaluation.

UTILIZATION OF PLANT INTRODUCTIONS

When a new species is adapted for commercial production, plant introductions are used directly as cultivars. The general procedure is to introduce the best cultivars from countries in which the crop is grown commercially, evaluate their performance, and release the best ones to farmers. Plant introductions also may include experimental lines from countries where plant breeding programs for the species are conducted.

Heterogeneous plant introductions provide an opportunity for selection of superior individuals that are useful as cultivars. The evaluation and release of selections have contributed important cultivars of a new crop.

Plant introductions are one source of parents for hybridization in a cultivar development program (Chap. 10). A plant breeder responsible for cultivar development is interested in the ease and success with which genes can be transferred among genotypes. Scientific names provide a means of understanding the taxonomic relationship among plants that can be helpful as a starting point for selecting species from which useful genes may be extracted. However, the ultimate value of a species as a source of useful characteristics requires an understanding of the feasibility of gene transfer to cultivars of the cultivated species. In recognition of this fact, Harlan and de Wet (1971) proposed that species of plants be grouped into one of three gene pools.

1. *Primary gene pool:* The primary gene pool includes the cultivated species of a crop and related species from which useful genes can be most readily obtained for a cultivar development program. Species in the primary gene pool can be readily crossed, chromosome pairing during meiosis in F_1 plants is normal, and F_2 seed is produced. The segregation of genes in populations obtained from F_1 plants is predominantly normal.
2. *Secondary gene pool:* Species in the secondary gene pool include those from which genes can be transferred to the cultivated species, but not without difficulty. When genotypes of the cultivated species are crossed to those in the secondary gene pool, hybrid seed can be obtained, but the F_1 plants may be weak and difficult to maintain, chromosome pairing during meiosis in the hybrids may be poor or not occur at all, and the

hybrids tend to be sterile. Progeny that are obtained from hybrid plants may not exhibit normal gene segregation, and recovery of segregates with the desired genes from a species of the secondary gene pool may be difficult.

3. *Tertiary gene pool:* Gene transfer from a species in the tertiary gene pool to the cultivated species in the primary gene pool requires special techniques or may not even be possible with the techniques currently available. Crosses can be made between some species in the tertiary and primary pool, and fertilization may take place to produce a hybrid embryo. If mature hybrid seed cannot be obtained, it may be possible to use embryo culture to obtain a viable hybrid plant. If hybrid seed is obtained, the hybrid plant may die prematurely or be completely sterile. Grafting or tissue culture may be required to secure a viable hybrid plant. Chromosome doubling may be required to obtain some fertility in the hybrid.

Of the three sources of genes, the primary gene pool receives the greatest attention for cultivar development. A plant breeder will rely on genotypes of the cultivated species as the primary source of genetic variability. When a character is not available in cultivars or plant introductions of the cultivated species, related species in the primary gene pool will be evaluated for the characteristic. The related species in the primary gene pool may include the wild progenitor of the crop or weedy species. The wild and weedy species often have characteristics that are unacceptable in cultivars of the crop, including shattering, lodging susceptibility, colored seeds, and small seed size. Developing a cultivar that has a desirable characteristic from a wild or weedy species generally involves multiple backcrosses to a recurrent parent of the cultivated species. Linkage between genes controlling the desirable characteristic and those controlling undesirable ones may make the transfer difficult.

The secondary gene pool generally is not considered as a source of genes for cultivar development unless the desired genes are not available in the primary gene pool. Some genes that have not been found in the primary gene pool, but are present in the secondary gene pool, have proven to be useful in the improvement of cultivated species, particularly for pest resistance. The breeder who intends to use genes from a species in the secondary pool must consider such barriers as lack of vigor and a high degree of sterility in hybrid plants. In addition, the species would likely have numerous unacceptable characteristics that would have to be eliminated through repeated backcrosses to the cultivated species.

Use of genes from the tertiary gene pool generally is only considered if a characteristic is lacking in the primary and secondary pools. The breeder has to be willing to spend a considerable amount of time making the gene transfer. Specialized training might be required to obtain the skills needed to carry out certain procedures involved with gene transfer.

It should be emphasized that extensive experimentation is required to determine the species that fit in each of the gene pools. This is particularly true of the tertiary gene pool. Relatively few persons specialize in the basic research

necessary to evaluate the feasibility of gene transfer from species that are not closely related to the cultivated one. Expanding research in tissue culture and molecular biology may expand the usable tertiary gene pool of crop species in the future.

CONSEQUENCES OF INSUFFICIENT GENETIC DIVERSITY

Two aspects of genetic diversity influence how cultivars are developed, how they are used in agricultural production, and what the consequence may be if sufficient diversity is lacking. One aspect is the genetic vulnerability of the crop to an unexpected production problem. The second relates to the continued improvement of cultivars for quantitative characters, such as yield.

Genetic Vulnerability

Genetic vulnerability refers to the possibility that an unexpected problem could cause a major loss in the production of most or all cultivars of a crop. This possibility was highlighted by the epidemic of southern corn leaf blight that endangered maize production in the United States during the early 1970s. Hybrid seed maize was produced in the 1960s by use of cytoplasmic-genetic male sterility. All of the female parents used to produce hybrid seed maize had male-sterile cytoplasm derived from the same source, referred to as T cytoplasm. As a result, all maize hybrids, regardless of their diversity for nuclear genes, were related by a common cytoplasm. A race of the southern corn leaf blight developed that had the ability to attack maize plants with T cytoplasm, which meant that the entire U.S. maize crop was vulnerable to the pathogen. Hybrid seed companies rapidly increased seed of female parents with male-fertile cytoplasm (B lines) that were not susceptible to the pathogen. The female parents with male fertility were emasculated by detasseling in hybrid seed production fields. The conversion to male-fertile cytoplasm was rapid and loss from the disease was minimal. Nevertheless, the situation made the public aware of the danger caused by lack of genetic diversity. A subsequent study conducted by the National Academy of Science (1972) on the potential genetic vulnerability of major crops grown in the United States found most major crop cultivars to be genetically related and potentially vulnerable.

Limitation of Genetic Improvement

Insufficient genetic diversity in the parents used to form populations by hybridization may lead to a reduction in genetic variability for quantitative characters. As a result, improvement of the character may be difficult or impossible to achieve. This potential consequence of limited genetic diversity can be more difficult to evaluate and to overcome than that of genetic vulnerability. Many factors affect the degree of success from breeding in a crop, and it is difficult

to identify clearly the effects of any one factor. It is difficult to determine whether a yield plateau has been reached in a crop, because yield improvement often does not occur at a constant rate. Breeders commonly conduct selection for several years before identifying a genotype that shows significant yield improvement. Because yield improvement often is sporadic and unpredictable, many years with no yield increase may be needed to be certain that a yield plateau has been reached. It also is important to determine whether a period without yield improvement is a result of the lack of genetic variability or due to insufficient emphasis on selection for yield. A breeding program that emphasizes improvement of characters other than yield can create an apparent yield plateau by restricting the parents that are used for crossing. Simultaneous selection for several characters also can restrict yield improvement, even though sufficient genetic variability for yield may be present.

MINIMIZING GENETIC VULNERABILITY

Systematic reduction of genetic vulnerability involves three interrelated steps: (a) the monitoring of pests or any other production problems that have the potential of becoming a threat to crop production, (b) the development of cultivars that will not be seriously influenced by the potential problem, and (c) the use of these cultivars for crop production.

Monitoring of Potential Problems

The systematic monitoring of crop pests is a worldwide project. Consideration must be given to new races of pests that are already in a country, and to new pests that may be introduced from other countries. The development of a cultivar with resistance to a pest can take many years. The early detection of a potential problem gives the breeder an opportunity to have appropriate cultivars ready, if they are needed.

Development of Cultivars with Increased Genetic Diversity

There is usually no simple way to increase the genetic diversity of cultivars of crops that already have been improved by plant breeding. The progeny of a cross between high-yielding parents are more likely to have superior yield than those from a cross between a high- and a low-yielding parent. For that reason, a plant breeder who is responsible for developing improved cultivars for yield and other quantitative characters in the immediate future will choose as parents those with the highest level of performance available. Plant introductions generally have poorer overall performance and may have major weaknesses not present in current cultivars. Populations developed from crosses between plant introductions and current cultivars generally are inferior to those available from crosses among

Table 11-3 Characteristics for Seed Yield of Five Soybean Breeding Populations with Different Levels of Plant Introduction (PI) Parentage

Population	Percent of PI Parents	Population $\bar{X}$ Yield (kg/ha)	Number of Superior Lines	Genetic Variability
AP1	100	2304b*	5	8,150 ± 4,783
AP2	75	2244c	1	24,234 ± 6,919
AP3	50	2293bc	7	29,185 ± 7,595
AP4	25	2338b	10	24,413 ± 6,942
AP5	0	2441a	22	13,926 ± 5,533

*Means followed by the same letter are not significantly different ($P > 0.05$) based on Duncan's multiple range test.

Source: Schoener and Fehr, 1979.

elite related parents. This has been illustrated by the performance of five populations of soybeans with different percentages of germplasm from plant introductions (Schoener and Fehr, 1979). The population with the greatest mean yield and the highest frequency of high-yielding segregates was the population that was developed from cultivars and elite breeding lines (Table 11-3).

A plant introduction may be desirable as a parent because it possesses an essential characteristic such as disease resistance. In this situation, it is a common practice to transfer the appropriate genes to local cultivars by backcrossing. Each generation of backcrossing reduces the likelihood that unique alleles for yield and other quantitative characters from the plant introduction will be present in the backcross progeny. It is doubtful, therefore, that plant introductions used as donor parents in a backcrossing program have contributed substantially to genetic variability for quantitative characters.

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CHAPTER TWELVE

Population Formation by Hybridization

The first step in a cultivar development program is to form a population with genetic variability for the characters of interest. This is done by hybridization of genetically different parents or by the use of mutagenesis. Population formation by hybridization will be the focus of this chapter.

TYPES OF POPULATIONS

The populations used for cultivar development range from a two-parent cross to a complex population involving several hundred parents. Their degree of homozygosity and homogeneity may vary considerably both within and among species. The population formed may be used for direct selection of a cultivar or may be utilized in a recurrent selection program.

Two-Parent Population

The simplest and most widely used population is formed by the mating of two parents, P1 × P2 (Fig. 12-1). The population is referred to as a two-parent cross, single cross, or two-way cross. The population may be used directly for selection or mated to other parents or populations.

Three-Parent Population

A three-parent cross is commonly referred to as a three-way cross. It is formed by mating a two-parent population to a third parent, (P1 × P2) × P3 (Fig.

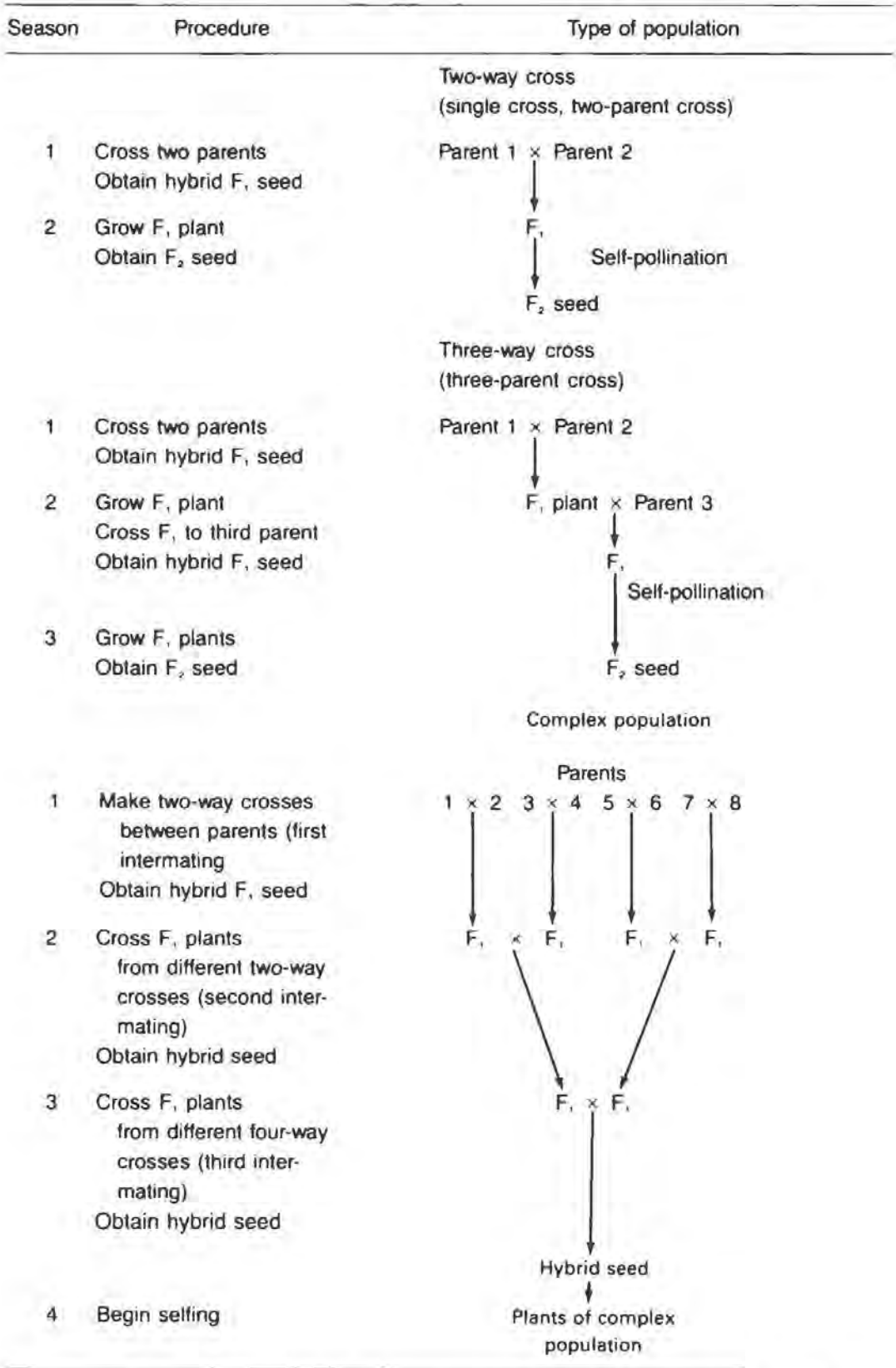


Figure 12-1 Development of a two-way cross, a three-way cross, and a complex population.

12-1). The plants of the two-parent population that are mated to the third parent may be in the F_1 or some later generation of self-pollination.

The three-parent cross is preferred by some breeders when one parent (P1) has a desirable character but is not adequately acceptable for other traits to be used in a successful two-parent cross. Parents P2 and P3 are desirable parents, except for the character to be obtained from P1. The three-parent cross results in a population with an average of 25 percent of the alleles from P1, 25 percent from P2, and 50 percent from P3. For example, large seed is preferred for certain uses of soybean seed. The market for large seed is minor compared with the amount of seed processed for protein and oil, for which seed size is of no consideration. The cultivars grown by farmers have relatively small seed and outyield large-seeded cultivars by over 15 percent. It would be desirable to combine the high yield of a small-seeded cultivar (P1) with the large seed weight of a lower yielding cultivar (P2). A two-parent population from the cross P1 × P2 has few, if any, segregates with adequate seed size (Bravo et al., 1981). Mating F_1 plants of the P1 × P2 cross to a third parent (P3) with large seed usually results in a population with an adequate frequency of large-seeded segregates.

Backcross Population

A backcross population is formed by mating two parents, P1 × P2, then crossing the population back to one of the two parents, (P1 × P2) × P2. The number of backcrosses can be one or more. Each backcross decreases by half the average number of alleles contributed to the population by the nonrecurrent parent: P1 × P2 = 50 percent P1, (P1 × P2) × P2 = 25 percent P1, [(P1 × P2) × P2] × P2 = 12.5 percent P1.

A backcross population is an alternative to a three-parent cross when one parent has a desirable character but is not adequately acceptable in other characters. Consider the example of seed size in soybeans that was used to illustrate a three-parent cross. An adequate frequency of large-seeded segregates in a population could be obtained from the three-parent cross (P1 × P2) × P3 or from the backcross (P1 × P2) × P2, where P1 is the high-yielding, small-seeded parent and P2 and P3 are large-seeded parents.

Four-Parent Population

A four-parent population can be formed by the mating (P1 × P2) × (P3 × P4). This mating is commonly referred to as a double cross or a four-way cross. In the mating of two single crosses, each of the parents contributes an average of 25 percent of the alleles in the final population.

A second method for the formation of a four-parent population is the mating [(P1 × P2) × P3] × P4. The genetic contribution of the parents to the final

population is not the same. The parents of the initial two-way cross, P1 and P2, have an average genetic contribution of 12.5 percent each, P3 contributes 25 percent, and P4 contributes 50 percent.

Complex Population

A complex population is one that is formed by hybridization of more than four parents. Complex populations were not widely used for selection in a cultivar development program in the past. They have been more common in recent years because of the interest in population improvement by recurrent selection.

The potential advantages of a complex cross compared with a single cross are that the number of possible alleles in the population for each locus increases with the number of parents used, and the probability of heterozygosity at multiple loci is greater. The first advantage relates to individual loci with two or more alleles, one of which is more favorable than the others. With two homozygous parents, only one allele can be contributed by each. For a quantitative character controlled by multiple genes, each with small effects, the breeder does not know which allele is present in a parent. In a single cross between homozygous parents, one, both, or neither parent may have the most favorable allele possible at a locus. The probability that at least one parent has the most favorable allele at each locus increases as the number of parents in the population increases.

The second advantage of a complex cross relates to the number of loci that will be heterozygous. The probability that homozygous parents will have different alleles at two or more linked loci increases as the number of parents of the population increases. Heterozygosity for linked loci is required before effective recombination can occur between them (Chap. 3).

PRINCIPLES IN THE FORMATION OF A COMPLEX POPULATION

Selection of a procedure to form a complex population depends on the importance that the breeder places on a number of factors. These factors include (a) the need to combine alleles from all parents into the members of a population, (b) the number of parents involved, (c) the genetic contribution of each parent to the population, and (d) the amount of time available to form the population. The impact of each of these factors on the genetic makeup of the population and the efficiency of its development will be considered.

Combination of Alleles from Different Parents

The alleles obtained by the members of a population is influenced by the number of generations of intermating conducted before selection is initiated. To provide the opportunity for a segregate to possess genes from every parent of a population,

two parents require a minimum of one intermating, three or four parents require two intermatings, five to eight parents require three intermatings, and so forth.

Number of parents	Number of intermating generations required
2	1
3–4	2
5–8	3
9–16	4
17–32	5

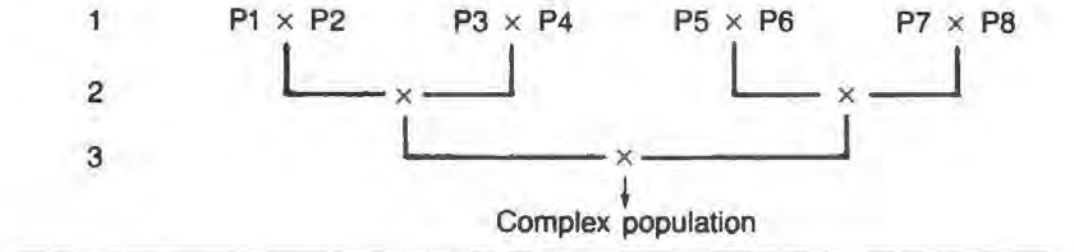
The genetic basis for the minimum number of generations of intermating required can be illustrated with alleles at four loci (A through D) for eight parents (1 through 8). The allele at a locus will be identified by the number of the parent from which it was obtained. For example, A1 is the A locus with an allele from parent 1. The mating arrangement used to illustrate the principle will be the convergent cross (Fig. 12-2).

Season 1: The matings involved and the genotype of the hybrids produced in the first generation of intermating would be

Parent 1 × parent 2 – hybrid 1–2 = A1A2B1B2C1C2D1D2
Parent 3 × parent 4 – hybrid 3–4 = A3A4B3B4C3C4D3D4
Parent 5 × parent 6 – hybrid 5–6 = A5A6B5B6C5C6D5D6
Parent 7 × parent 8 – hybrid 7–8 = A7A8B7B8C7C8D7D8

Season 2: In the second generation of intermating, the cross of hybrid 1–2 × hybrid 3–4 can produce an array of genotypes, one example of which is A1A3B2B4C1C3D2D4. The mating of hybrid 5–6 × hybrid 7–8 can produce genotypes such as A5A7B6B8C5C7D6D8. By the end of the second generation of intermating in season 2, an individual can possess alleles from four of the eight parents of the final population.

Figure 12-2 Complex population developed from eight parents by the convergent-cross procedure. (Courtesy of Harlan et al., 1940.)



Season 3: In the third generation of intermating, the cross of hybrid 1–2–3–4 × hybrid 5–6–7–8 can produce genotypes with alleles from all parents, such as A1A5B2B8C3C7D4D6.

Number of Parents Involved

The number of different alleles possible in a population theoretically increases with each additional parent used to form a population. An increase in the number of parents, therefore, enhances the possibility of increased genetic variance in the population. It often is difficult, however, to find a large number of parents that have an acceptable level of performance for the characters under selection. Out of a group of 40 genotypes available as parents, 10 may have superior performance for a character, 10 may be average, 10 may be below average, and 10 may be inferior. The breeder must decide if it is better to use the 10 superior parents to obtain a final population with a potential for high mean performance and limited genetic variability, or use more than 10 parents to increase potential genetic variability in the population with a sacrifice in its mean performance.

The number of parents used to form a population can influence the procedures available to carry out the required matings. The convergent-cross procedure can only be used with a multiple of 2^n parents: 4, 8, 16, 32, and so forth (Fig. 12-2). The choice between a complete or partial diallel design mating will be influenced by the number of parents involved relative to the resources available for hybridization. If resources were available to make 36 matings, the number of parents could not exceed six if a diallel design with reciprocal crosses and self-pollinations were preferred, could not exceed nine for a diallel design without reciprocals and selfs, and could not exceed 72 if each parent were crossed to one another in a partial diallel design.

Genetic Contribution of Each Parent

The potential advantage of mating genetically diverse parents is that each may contribute unique alleles, which when combined together may result in a superior individual. The theoretical advantage has seldom been realized for short-term improvement of cultivars for quantitative characters, such as seed yield. Populations in which highly productive cultivars contribute half the germplasm, and less productive plant introductions the other half, seldom produce a segregate that is superior to the most productive cultivar used as a parent. Breeders who are attempting to increase genetic diversity in breeding populations are faced with the choice between genetic variability and population performance. A population with broad genetic variability is of little value if the best segregates are inferior in performance to other available populations.

Some breeders choose to develop breeding populations in which certain

parents contribute less than 50 percent of the germplasm. To illustrate alternative mating designs that are used, highly productive parents will be designated by the letter A and less productive plant introductions with the letters PI (Fig. 12-3).

One alternative for varying the percentage of plant introductions in a population is by the relative frequency of A and PI parents used. For a convergent cross with eight parents, the percentage of PI parentage in the final population could be varied by the fraction of PI parents used: 1 PI/7A, 2 PI/6A, 3 PI/5A, 4 PI/4A, and so forth. A disadvantage of this procedure is that a low number of different PI parents will be involved when the desired percentage of PI germplasm is low. For example, a 2 PI:6A ratio has 25 percent PI germplasm, which is contributed by only two parents.

Some form of backcrossing can be used to include a large number of PI

Figure 12-3 Two procedures for complex population formation that permit the use of multiple plant introductions (PI) as parents with a limited percentage of the germplasm of each in the final population.

Season	% A germplasm	Matings
1	50	A1 × PI1 A2 × PI2 A3 × PI3 A4 × PI4 A5 × PI5 A6 × PI6
		↓ ↓ ↓ ↓ ↓ ↓
2	75	A1 × A2 × A3 × A4 × A5 × A6 ×
		↓ ↓ ↓ ↓ ↓ ↓
3	88	A1 × A2 × A3 × A4 × A5 × A6 ×
		↓ ↓ ↓ ↓ ↓ ↓
4	94	A1 × A2 × A3 × A4 × A5 × A6 ×
5	94	Diallel of the six backcross populations
6	94	Random mating of the hybrids from season 5
7	94	Random mating is continued for as many generations as desired

Season	% A germplasm	Matings
1	50	A1 × PI1 A2 × PI2 A3 × PI3 A4 × PI4 A5 × PI5 A6 × PI6
		↓ ↓ ↓ ↓ ↓ ↓
2	75	A2 × A3 × A4 × A5 × A6 × A1 ×
		↓ ↓ ↓ ↓ ↓ ↓
3	88	A3 × A4 × A5 × A6 × A1 × A2 ×
		↓ ↓ ↓ ↓ ↓ ↓
4	94	A4 × A5 × A6 × A1 × A2 × A3 ×
5	94	Diallel of the six populations
6	94	Random mating of the hybrids from season 5
7	94	Random mating is continued for as many generations as desired

parents and still minimize the percentage of PI germplasm in the final population. Two of the possible procedures are illustrated in Fig. 12-3. One procedure is to mate each A parent to a PI parent, backcross to the A parents until the desired level of A parentage is achieved, then intermate the backcross populations to form a single complex population. In the second procedure, the A parents are rotated for each backcross until the desired level of A parentage is achieved, then the backcross populations are intermated to form a single population. The advantage of the second procedure is that there can be recombination of genes from the different parents during backcrossing. In the first procedure, recombination only occurs between the A and PI parent in each separate backcross program.

Amount of Time Available

The amount of genetic improvement per year in a breeding program is strongly affected by the length of time required for completion of a cycle of selection (Chap. 17). This applies to direct selection of cultivars from a population or some type of recurrent selection program. One important variable in the number of years per cycle is the length of time required to form a new population. Breeders may prefer to conduct three or more generations of intermating before selection begins, but this may add 1 or more years to the length of a cycle. The breeder must decide if more genetic improvement per year will be achieved by providing additional opportunity for recombination before selection or by conducting more cycles of selection per unit time in populations formed with minimal intercrossing.

At the present time, most breeders prefer to conduct more cycles of selection than to spend time on additional generations of intercrossing, particularly when the parents are heterozygous. It is common in recurrent selection programs involving heterozygous plants or lines to form a population by crossing parents together in a series of single-cross matings, to bulk seed from each single cross to form one population, and to initiate selection without any additional generations of intermating. As a result, the members of the new population have alleles from only two of the parents, regardless of the number of parents used to form the population. Such a population is complex in the sense that alleles from many parents are present, but it is not complex with respect to the opportunity for recombination of alleles from different parents.

PROCEDURES USED TO FORM COMPLEX POPULATIONS

Breeders can use a number of procedures to form complex populations. The following discussion will be limited to procedures reported in the literature and those commonly used by plant breeders.

Convergent Cross

The alleles obtained by an individual in a population from a set of parents is a function of the number of parents involved and the mating arrangement used to form the population. One mating procedure that provides an equal probability for alleles from each parent to be present in an individual was proposed by Harlan and colleagues (1940) as a "systematic series of bridging crosses," later referred to as a convergent cross (Fig. 12-2).

The convergent cross provides the opportunity for alleles of each parent to be present in segregates of the final population. It is seldom used, however, because of several disadvantages: (a) The number of parents is restricted to a multiple of 2^n: 4, 8, 16, 32, 64. (b) Recombination of genes from some parents is not possible until the final population is formed. For example, consider the procedure for eight parents illustrated in Fig. 12-2. Genes from parent 1 have no opportunity to recombine with those from parents 7 and 8 until the third season of intermating. (c) The number of seasons required to form the population often exceeds the length of time a breeder is willing to spend on population formation.

Diallel Mating Design with Reciprocal Crosses and Self-Pollinations

A diallel design with reciprocal crosses and self-pollinations is required to achieve Hardy–Weinberg equilibrium in a population (Chap. 3). Use of the procedure in applied breeding programs is limited to natural hybridization in certain open-pollinated populations. One example would be the mating of selected individuals in a program of recurrent phenotypic selection. Parents (plants) are selected from among male-fertile individuals before pollination, and selected parents are subjected to both self- and cross-pollination. A diallel design with reciprocals and selfs is not possible when self-incompatibility or male sterility reduces or eliminates the probability of self-pollination, or when male sterility prevents the possibility of reciprocal crosses.

The advantages of a diallel design mating with reciprocal crosses and self-pollinations are (a) the number of parents in the population is not restricted, (b) each parent has the opportunity to mate and recombine with every other parent, and (c) Hardy–Weinberg equilibrium can be achieved in the populations. However, the procedure is not considered practical for applied breeding programs that utilize artificial hybridization for several reasons. (a) Self-pollinations do not permit recombination of genes between parents. (b) Crosses in only one direction are as effective for recombination as reciprocal crosses between two parents. (c) An extensive number of matings are required (p^2), which limits the number of parents (p) that can be considered.

Diallel Mating Design without Reciprocal Crosses and Self-Pollinations

A common procedure for mating a limited number of parents to form a complex population is to use a diallel design without reciprocal crosses and self-pollinations. This type of diallel mating design provides the opportunity for genes of each parent to recombine with those of every other parent. If sufficient generations of intermating occur, individual members of a population will have the opportunity to obtain alleles from every parent. Without reciprocal crosses and self-pollinations, Hardy–Weinberg equilibrium will be approached but never achieved. This is not considered a problem for cultivar development programs.

The advantages of a diallel mating design without reciprocal crosses and self-pollinations are (a) the number of parents used to form the population is not restricted, (b) each parent has the opportunity to mate and recombine with every other parent, (c) the method applies to open-pollinated populations that involve self-incompatibility or genetic male sterility, and (d) the method involves fewer matings than necessitated by a diallel design with reciprocal crosses and self-pollinations. The primary disadvantage is that the large number of matings required, $p(p - 1)/2$, often limits the number of parents that can be included for artificial hybridization.

Partial Diallel Mating Design

The mating of each parent to some, but not all, of the other parents in a partial diallel design is commonly used in the formation of a complex population. It is used with artificial hybridization whenever the number of parents is too great to accomplish a complete diallel. A partial diallel is operative in an open-pollinated population whenever the number of seeds produced by a parent is less than the number of parents with which it must mate to achieve a diallel.

The advantages of a partial diallel design are (a) the number of parents in the population is not restricted, (b) it can be used with open-pollinated populations that involve self-incompatibility or male sterility, and (c) it involves fewer matings than necessitated by a diallel. The primary disadvantage of a partial diallel is that each parent does not have the opportunity to mate and recombine with every other parent.

Combination of the Diallel and Partial Diallel Mating Designs

Both the diallel and partial diallel designs are commonly used in the same program to form a complex population. The matings may be accomplished by artificial hybridization, open pollination, or a combination of the two. A combination of procedures is used to permit the greatest amount of recombination possible within

the resources available for hybridization. Many combinations of the diallel and partial diallel mating designs are possible. A few of the more common ones are the following.

1. *Procedure 1:*

Season 1: A diallel without reciprocal crosses and self-pollinations is made to form $p(p - 1)/2$ single-cross populations.
Season 2: A partial diallel is used to intercross the single-cross populations formed in season 1.
Season 3: Hybrid seeds from season 2 are bulked and planted as one population. A partial diallel consisting of plant-to-plant crosses is used for the third generation of intermating.

2. *Procedure 2:*

Season 1: The number of parents is too large to permit a diallel; therefore, each parent is mated to one other in a partial diallel.
Season 2: There are $p/2$ single-cross populations from season 1. The number is small enough to permit a diallel mating of the populations without reciprocals and self-pollinations in season 2.
Season 3: A bulk of hybrid seed from season 2 is planted. A partial diallel consisting of plant-to-plant crosses is used for the third generation of intermating.

3. *Procedure 3:*

Season 1: A partial diallel is used to mate a large number of parents.
Season 2: Hybrid seed from season 1 is planted as a bulk in isolation. Natural hybridization is used for the second generation of intermating.
Season 3+: All subsequent generations of intermating are conducted in the same manner as described for season 2.

PLANTING ARRANGEMENTS FOR POPULATION FORMATION BY ARTIFICIAL HYBRIDIZATION

After the parents have been chosen and the mating procedure established, the breeder must select an appropriate planting arrangement when artificial hybridization is conducted in the field. Each arrangement has advantages and disadvantages, so the breeder must select the one that will be most efficient for the circumstances encountered and the resources available.

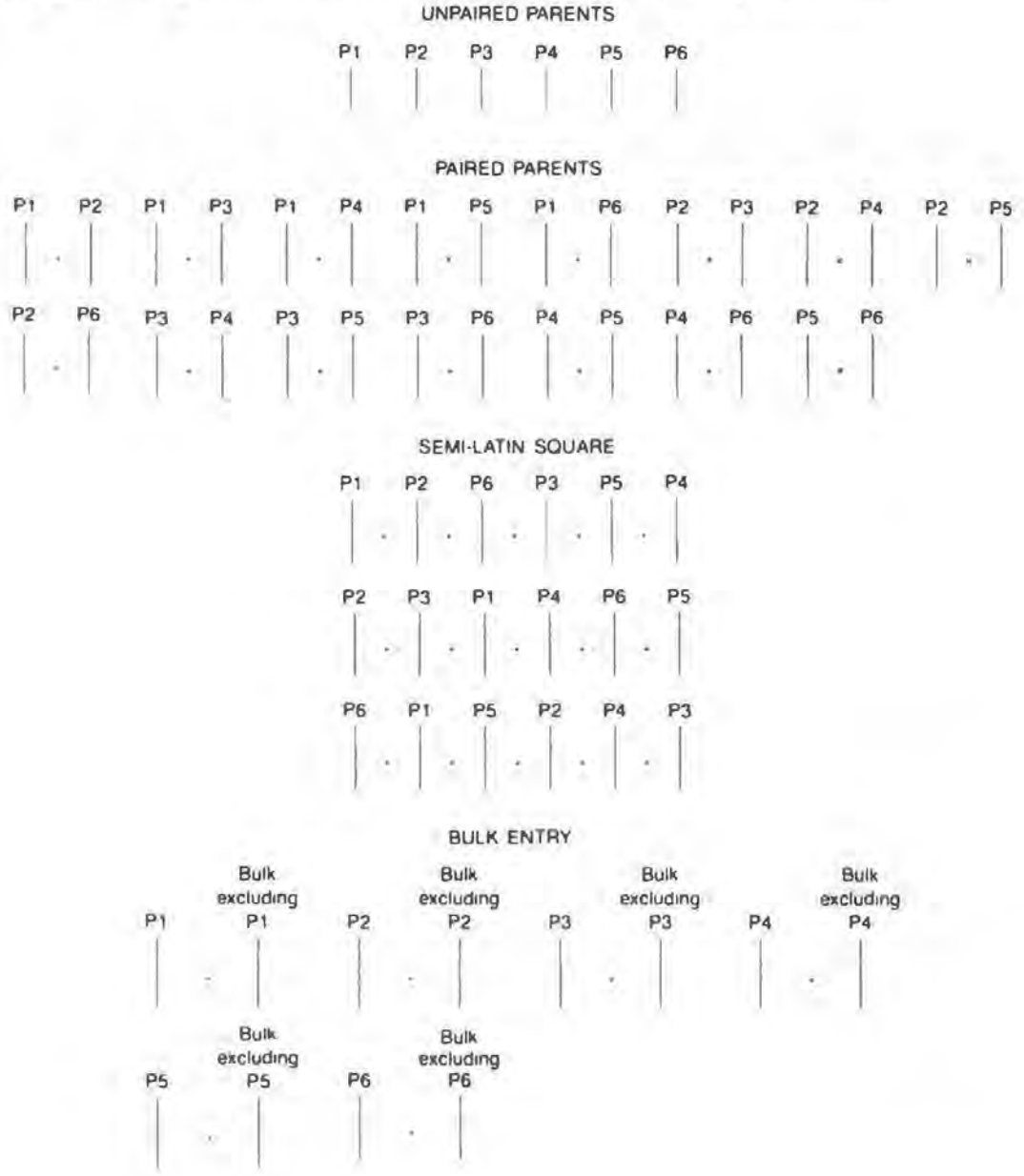
Diallel Matings

Alternative planting arrangements for artificial hybridization can best be described by first considering their use in making a diallel design of six parents

without reciprocal crosses and self-pollinations. The four arrangements to be considered are unpaired parents, paired parents, a semi–latin square, and a bulk-parent method.

Unpaired Parents. A common planting arrangement for any type of mating program is the use of unpaired parents (Fig. 12-4). Each parent is planted once, regardless of the number of parents with which it will be crossed. The parents may be grown in a special area designated for crossing or may be located in different parts of a field.

Figure 12-4 Four planting arrangements that can be used for a diallel mating design of six parents without reciprocal crosses and self-pollinations.

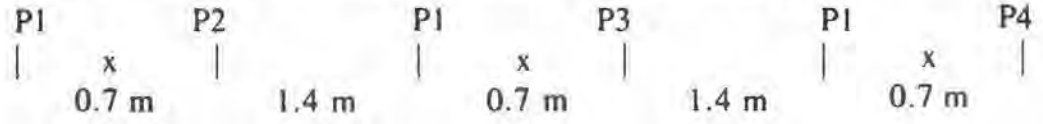


The advantages of the unpaired-parents method are that (a) it requires the least space and parent seed of the four arrangements because there is only one row of each parent for each planting date, (b) the decision on which parents to mate can be made after planting, (c) the parents of each mating are known, and (d) it can be used with any number of parents.

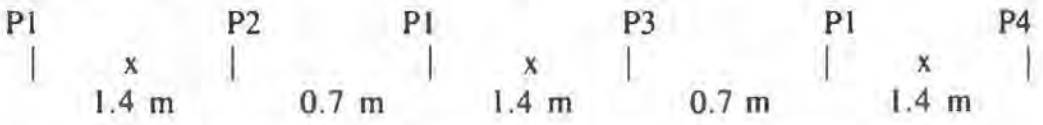
One disadvantage of unpaired parents is that more time is required per mating during hybridization than for any of the other three arrangements. The availability of suitable female flowers and pollen must be determined for each pair of parents by walking from one area of the field to another. Pollen must be transported between parents. The male parent must be identified for each cross made. An inventory of number of crosses made and success of a particular mating can be time-consuming. A second disadvantage is that there is a greater chance that the wrong mating will be made accidentally, particularly by inexperienced persons.

Paired Parents. Some of the disadvantages of the unpaired-parents method can be overcome by planting adjacent to each other the pairs of parents to be mated (Fig. 12-4). When the paired parent arrangement is used, breeders employ various techniques to facilitate movement through the field during hybridization and to prevent persons from getting in the wrong place and making an incorrect mating.

1. The spacing between rows of two parents to be mated can be different than the spacing between adjacent rows of other parents. For example, the distance between rows as marked by a commercial planter may be 0.7 m. The breeder may plant parents to be mated 0.7 m apart, and skip a row on either side of the pair to provide a 1.4-m space.



Conversely, the breeder may choose to mate the parents on either side of the 1.4-m space, where it is easier to walk.



2. A string may be hung between pairs of rows that are to be mated. The string serves as a barrier to prevent persons from mating the wrong pairs of rows.
3. Stakes of the same color may be placed at the beginning of rows to be mated. Colors are alternated across the field to differentiate pairs of parents to be mated.

The advantages of the paired-parent arrangement are that (a) less time is required per mating during hybridization than for unpaired parents, (b) there is less chance that the wrong mating will be made than with unpaired parents, (c)

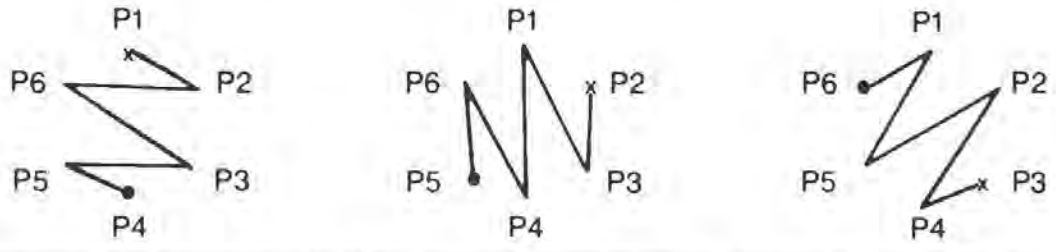
the parents of each mating are known, and (d) the method can be used with any number of parents. The disadvantages are that (a) it requires the most space and parent seed of the four arrangements because the number of rows required for planting is twice the number of matings to be made and (b) the decision on parents to be mated must be made before planting.

Semi–latin Square. The disadvantage of the paired-parent arrangement for making a diallel is the large amount of space and parent seed required. To reduce the space and parent seed requirement, Fehr and Ortiz (1975) described the use of the semi–latin square to make a diallel when an even number of parents was involved. The arrangement also could be called half of a symmetric latin square or a partial latin square.

The systematic planting order for the semi–latin square is determined by the procedure illustrated in Fig. 12-5.

Figure 12-5 Arrangement of parents for a diallel design of six parents using the semi–latin square arrangement. The number of columns (mating units) utilized is equal to half the number of parents in the diallel.

P1	P2	P3
X	X	X
P2	P3	P4
X	X	X
P6	P1	P2
X	X	X
P3	P4	P5
X	X	X
P5	P6	P1
X	X	X
P4	P5	P6
Order of first column (mating unit)	Order of second column (mating unit)	Order of third column (mating unit)



x = first parent in the column.
• = last parent in the column.

1. A parent is assigned to the first position in half the number of the columns normally used in a latin square. It makes no difference which half of the parents are used to begin the columns or to which column they are assigned.
2. The order of parents in each column is determined by writing the numbers of the parents in consecutive order in a circle, one circle for each column. For a given column in the latin square, a line is drawn among the numbers in a circle beginning with the first parent in the column and ending with the last. The line proceeds from the parent heading the column to the first parent in the clockwise direction, to the first parent in the counterclockwise direction, to the second parent in the clockwise direction, to the second parent in the counterclockwise direction, to the third parent in the clockwise direction, to the third parent in the counterclockwise direction, and so on until all of the parents have been assigned a position in the column. The order of parents in the line is recorded in the appropriate column of the semi–latin square.

For planting and hybridization, each column is an independent mating unit. The mating units can be planted in separate fields if necessary without any problem. At the time of hybridization, adjacent parents in a mating unit are crossed to each other in any direction preferred. The parent at the head of the mating unit and the one at the end have only one parent adjacent to them; therefore, they are involved in only one mating. Those two parents are crossed in one of the other mating units. All other parents in a mating unit have an adjacent parent on either side and are involved in two matings.

The advantages of the semi–latin square arrangement are (a) less time is required per mating during hybridization than for unpaired parents, (b) there is less chance that the wrong mating will be made than with unpaired parents, (c) less space and parent seed are required than for paired parents, and (d) the parents of each mating are known. The disadvantages of the semi–latin square are (a) it requires more space and parent seed than unpaired-parent or bulk-parent arrangements, (b) the decision on parents to be mated must be made before planting, and (c) it can only be used with an even number of parents.

Bulk Parent. The bulk-parent arrangement can be used to form a diallel when knowledge of the parentage and frequency of each single-cross mating is not important. The arrangement also has been called the Irish and bulk-entry method (Stuber, 1980).

A row of each parent is planted adjacent to a row that is a bulk of all other parents in the diallel (Fig. 12-4). For example, if 6 parents are in the diallel, parent 1 is grown adjacent to a bulk of parents 2 to 6. All plants in the bulk row are mated to parent 1. Another row contains parent 2, adjacent to which is a bulk of parents 1 and 3 to 6. All plants in the bulk row are mated to parent 2. The plants in the bulk row are not identified; therefore, only one parent of each mating is known.

The techniques used by breeders to ensure that the correct pairs of rows are mated, as discussed for paired parents, apply also to the bulk-parent arrangement. In addition, the row of each pair that is the single parent must be differentiated from the bulk row because each plant of the bulk row must be used for crossing, *if possible*.

Although both parents of a mating are not known with the bulk-parent arrangement and some single-cross matings could be accidentally excluded, the arrangement provides two opportunities for each mating to occur. For example, if 20 parents are in the diallel, a row of parent 1 would be mated with a bulk containing parent 20, and a row of parent 20 would be mated with a bulk containing parent 1.

The bulk-parent arrangement used for artificial hybridization is similar in principle to the polycross method used to intermate parents by natural hybridization. In both methods, only one of the parents of a mating is known. The population, formed by bulking similar quantities of hybrid seed from each of the known parents, is assured of containing genes from every parent, but not necessarily in equal frequency.

The advantages of the bulk-parent arrangement are (a) less space and parent seed are required than for the paired-parent and semi–latin square arrangements, (b) less time is required per mating during hybridization and there is less chance of making the wrong mating than with unpaired parents, and (c) it can be used with any number of parents. The disadvantages are (a) only one parent of the hybrid seed is known, (b) the decision on parents to be mated must be made before planting, and (c) it requires more space and parent seed than unpaired parents.

Partial Diallel Design and Less Systematic Matings

Unpaired and Paired Parents. The unpaired- and paired-parent arrangements can be used to make a partial diallel with any number of matings per parent. They also can be used for any other type of mating that is desired. The advantages and disadvantages of the two planting arrangements described for the diallel also apply to their use for any other type of mating.

Circular Cross. A circular-cross arrangement can be used to form a partial diallel in which each parent is mated to two others. The procedure has also been referred to as the chain cross (Stuber, 1980). Adjacent parents are mated to each other, and the first parent in the sequence is mated to the last one. With the use of multiple circles, the number of single-cross matings for each parent can increase by multiples of two. For example, the two following arrangements each would provide two matings for each of the six parents.

$$(P1 \times P2 \times P3 \times P4 \times P5 \times P6) \times$$

or $$(P1 \times P3 \times P5 \times P2 \times P6 \times P4) \times$$

The advantages of the circular-cross arrangement are that (a) less time is required per mating during hybridization than for unpaired parents, (b) there is less chance that the wrong mating will be made than with unpaired parents, (c) the parents of each mating are known, (d) the method can be used with any number of parents, and (e) it requires less space and parent seed than for paired parents. The disadvantages are (a) it requires more space and parent seed than the unpaired-parents method and (b) each circle can only be used to mate each parent to two others. Additional circles would have to be used to increase the number of matings with each parent in the partial diallel.

POLYCROSS PROCEDURE

Population formation may include one or more generations of natural hybridization in species with appropriate mechanisms for open-pollination. The polycross is a widely used procedure for intercrossing parents by natural hybridization.

The polycross is a method for intercrossing parents of vegetatively propagated species with mechanisms that prevent or minimize self-pollination. Self-incompatibility in bromegrass minimizes the frequency of self-pollination. In alfalfa, a membrane over the stigma prevents self-pollination. The membrane is broken to permit cross-pollination when the flower is tripped by an appropriate insect.

The objectives of a polycross procedure are to intercross the parents as equally as possible and obtain a similar genetic contribution from each parent in the population that is formed. Several principles related to natural intercrossing must be considered.

1. Parents must be flowering at the same time for effective crossing to occur. This may restrict the range in maturity of parents that can be used in the polycross.
2. Parents adjacent to each other are most likely to be intercrossed. Replication and randomization of parents provide a reasonable assurance of random mating.
3. The method of sampling hybrid seed from the parents may influence the genetic contribution of each to the population.

Parent Arrangements

Two experimental designs normally associated with statistical analyses are used for polycrosses, the latin square and the randomized complete-block. The designs are used to arrange parents in a manner that will maximize intercrossing by placing the parents adjacent to each other as frequently as possible.

A latin square is a design in which each parent (entry) must occur in each row and column of plots in an experiment (Fig. 12-6). As a result, the number of replications of each parent is equal to the number of parents, and the number of plots is the square of the number of parents. There are four replications with four parents (16 plots), eight replications with eight parents (64 plots), and 16 replications with 16 parents (256 plots). A breeder can use two or more latin squares for a set of parents to increase replication. Four latin squares of four parents would provide 16 replications, and two latin squares of seven parents would result in 14 replications. Randomization of entries in a latin square is ensured by randomizing the order of rows, columns, or both. The advantage of the latin square arrangement compared with the randomized complete-block is that each parent occurs adjacent to every other parent somewhere in the latin square. Its disadvantage is that for a large number of parents, the number of replications required may be greater than desired.

The randomized complete-block is a design in which each parent is randomly assigned to a plot within each replication (Fig. 12-6). The advantage of the randomized complete-block design compared with the latin square is that the number of replications can be any number desired, which is particularly important for a large number of parents. Its disadvantage is that the proximity of parents to each other cannot be ensured. The difference in proximity of parents in the two designs is illustrated in Fig. 12-6.

A polycross is sometimes used to describe an unreplicated planting of parents that are open-pollinated. A group of plants in a field may be evaluated for a character and the unacceptable ones discarded. The selected ones are allowed to open-pollinate to form the new population. The advantages of an unreplicated

Figure 12-6 Latin square and randomized complete-block designs for a polycross of five parents with a total of 10 replications.

Latin square 1

P1	P2	P5	P3	P4
P3	P4	P2	P5	P1
P5	P1	P4	P2	P3
P2	P3	P1	P4	P5
P4	P5	P3	P1	P2

Latin square 2

P1	P2	P5	P4	P3
P2	P3	P1	P5	P4
P4	P5	P3	P2	P1
P5	P1	P4	P3	P2
P3	P4	P2	P1	P5

Randomized complete-block

Replication									
1	2	3	4	5	6	7	8	9	10
P5	P1	P5	P4	P2	P4	P1	P5	P2	P5
P1	P2	P1	P1	P1	P1	P4	P3	P1	P4
P3	P5	P4	P3	P5	P5	P5	P2	P3	P2
P2	P3	P2	P2	P4	P2	P3	P4	P5	P3
P4	P4	P3	P5	P3	P3	P2	P1	P4	P1

arrangement are (a) hybridization may be accomplished during the same season that selection is practiced, which reduces the number of years for population formation, and (b) much less labor is involved than with a latin square or randomized complete-block design. The disadvantage is that the mating of parents can be quite unequal, because the parents in closest proximity to each other will cross more frequently than those spaced farther apart.

Bulking Hybrid Seed

The genetic contribution of each parent to the population obtained from a polycross can be influenced by the procedure used to harvest and bulk seed from the parents. Three procedures can be used, each with inherent advantages and disadvantages.

1. The seed from all parents may be bulked without regard to the amount of seed produced by each parent. The advantage of this procedure is that it requires less labor than the following two procedures. The disadvantage is that the genetic contribution of each parent to the population may be markedly different if the amount of seed produced by each parent is different.
2. The seed from each plot (replication) of each parent may be harvested separately. The seed from plots of each parent is bulked, regardless of the amount of seed per plot. An equal amount of seed from the bulk of each parent is mixed together to form a single population. The advantage is that the genetic contribution of each parent to the final population is controlled better than with the first procedure, because the number of seeds from each female parent is the same. The disadvantages are that this procedure requires more labor than the first procedure and does not control the genetic contribution of the male parent as effectively as the third procedure.
3. The seed from each plot (replication) of each parent is harvested separately. An equal quantity of seed from each plot of a parent is bulked. An equal amount of seed from the bulk of each parent is mixed together to form a single population. The advantage is that the genetic contribution of each parent to the final population is controlled better than for the other two procedures. In each replication, the parents most adjacent to each other will cross most frequently. Sampling of an equal quantity of seed from each replication of a parent means that the genetic contribution of adjacent male parents, which differ in each replication, is similar. The disadvantage is that it requires more labor than the other two procedures.

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CHAPTER THIRTEEN

Techniques for Artificial Hybridization

Successful and efficient artificial hybridization is an important aspect of plant breeding. It requires knowledge of the reproductive structure and development of the species, the conditions needed to promote flowering and seed development, and the procedures for emasculation and pollination. There is extensive variation among species for all aspects of artificial hybridization; therefore, it is only possible in this chapter to review some of the general principles that are involved. Specific procedures used in artificial hybridization of plant species are provided in Bassett (1986), Fehr and Hadley (1980), and Fehr (1987).

REPRODUCTIVE STRUCTURE AND DEVELOPMENT

The location of the male and female reproductive organs and the timing of their development are major considerations in artificial hybridization. The flowers may be bisexual and contain both the male and female organs, or the male and female organs may occur in separate unisexual flowers (Lersten, 1980) (Chap. 2). In monoecious species, the male and female organs are located in separate unisexual flowers on the same plant. In dioecious species, unisexual male and female flowers are located on separate plants. Lengths of the styles and stamens are similar in most species with bisexual flowers; however, there are species with pin flowers, in which the style is longer than the stamen, or thrum flowers, in which the stamen is longer than the style.

The female organ in a bisexual flower generally is receptive to pollination at the time pollen is shed, and fertile male pollen must be eliminated to avoid self-pollination. The process, referred to as emasculation, may require manual removal of the anthers, or a more indirect procedure may be adequate.

The reproductive organs of bisexual flowers in leguminous species may be protected by a calyx, corolla, or both. In grass species, the reproductive organs of bisexual flowers may be enclosed by glumes, a lemma and palea, or both. The protective structure surrounding the reproductive organs may have to be partially or entirely removed during artificial hybridization.

The number of anthers in a flower varies among most species from 3 to 10. The anthers within a flower may be independent, fused, or both. Their size ranges from less than 1 mm to several millimeters in length. Procedures for manipulation of the anthers during emasculation and pollen collection vary widely among species.

FLORAL INDUCTION

The simultaneous flowering of parents to be mated and the suitability of the flowers for successful artificial hybridization are major considerations in artificial hybridization. The environmental conditions that prompt floral induction and adequate flower development may vary widely among genotypes within a species, as well as among species. The primary environmental factors considered for the development of suitable flowers and successful seed set are light, temperature, moisture, and soil fertility (Major, 1980).

Light

Induction of flowering in many species is controlled by the duration of the dark period. By tradition, however, it is referred to as a day-length rather than a night-length response. The response of genotypes to day length is divided into three categories: short-day, long-day, and day-neutral. A short-day plant flowers when the day length is equal to or less than a critical duration, referred to as the critical photoperiod. A long-day plant flowers when the day length is equal to or greater than the critical photoperiod. Floral induction of a day-neutral plant, also referred to as a day-length-insensitive plant, is not controlled by day length.

Genotypes within a species often vary in the critical day length required to induce flowering. Their response generally reflects the day-length conditions present during the growing season in the geographical area to which they are adapted. A genotype of a short-day species grown at a low latitude where day length is short would have a much shorter critical photoperiod than a genotype adapted to higher latitudes. The reverse would be true for a long-day species.

Photoperiod, light level, and quality can influence floral induction of some species when artificial lighting is used to delay or induce flowering. Light level refers to the intensity present at the plant surface. Flowering generally can be delayed or induced at light levels far below that of sunlight. Light quality refers to the relative amount of different wavelengths emitted by the light source. An

incandescent lamp has a higher percentage of the red wavelength than does a fluorescent lamp. There is variation among genotypes within a species and among species for the quality of light that will most effectively control flowering; therefore, a single type of lamp is not suitable for all species.

Experiments on the effect of light on flowering generally have considered only the presence of flowers, regardless of their suitability for artificial hybridization. The use of extremely short days for a short-day species may promote flowering, but the flowers may be too small to manipulate for artificial hybridization.

Temperature

A well-known effect of temperature on flowering is the vernalization requirement of some species. Vernalization is the exposure of plants to a period of cold temperature to enhance their sensitivity to stimuli that promote flowering. Winter wheat is an example of a species with a vernalization requirement. When sown in the fall and exposed to winter temperatures, the species will flower and mature during the following summer. The same wheat sown in the spring will not flower normally or produce adequate seed in the summer.

The influence of temperature on the induction of flowering can involve minimums, maximums, cumulative amounts of heat, or a combination of the three factors. In maize and sorghum, for example, the expected flowering date of a genotype can be defined by the number of growing-degree-days that it must accumulate during the growing season. The number of growing-degree-days for a 24-hour period is computed from the equation [(daily maximum temperature + daily minimum temperature)/2] − 10°C. Temperatures above the maximum or below the minimum do not enhance the initiation of flowering. In maize and sorghum, 30°C is considered the maximum and 10°C the minimum temperature. If the maximum for a day were 33°C and the minimum 8°C, there would be [(30 + 10)/2] − 10 = 10 growing-degree-days. If the maximum daily temperature were 29°C and the minimum 12°C, the number of growing-degree-days would be [(29 + 12)/2] − 10 = 10.5.

Temperatures that are adequate for natural hybridization may not be suitable for artificial hybridization. At an excessively low or high temperature, pollen shed in bisexual flowers may be too limited for artificial hybridization, even though there are adequate amounts of pollen for natural self-pollination.

Moisture

Inadequate or excessive moisture during flowering can reduce the success of artificial hybridization. Whenever possible it is desirable to control by irrigation the availability of soil moisture. The relative humidity of the air can influence the tendency of exposed flowers to become excessively dry and abort, regardless

of the soil moisture available. In some species, the relative humidity of the air surrounding a flower that is used as a female in artificial hybridization will be kept as high as possible by enclosing the pistil with the outer parts of the flower or placing the entire flower in an appropriate bag.

Soil Fertility

The principal objective in soil fertilization is to obtain plants that are vigorous and healthy. Such plants are more likely to have normal flower development, pollen shed, and seed set, which enhance the success of artificial hybridization. Nitrogen levels are important for nonleguminous species or for legumes that are not properly inoculated. Phosphorus and potassium levels should be maintained at adequate levels, and special nutrients should be provided as needed.

TECHNIQUES FOR ARTIFICIAL HYBRIDIZATION

Synchronization of Flowering

The first step in artificial hybridization is to obtain receptive flowers of the female parent at the time when suitable quantities of fertile pollen are available from the male (Fehr, 1980; Major, 1980). Special techniques may be required to synchronize the flowering of parents that normally would not bloom at the same time.

Multiple Planting Dates. The most common method for synchronizing flowering is to plant one or more of the parents on different dates. Three different techniques are used in the field depending on the planting arrangement employed: (a) A separate block is used for each date of planting for unpaired parents. (b) The row for each parent is subdivided into sections for different planting dates. (c) Plantings for different dates are interspersed within a row.

The advantage of using separate blocks for each planting date is the ease of mechanical land preparation and planting. A heavy rain after one planting date could necessitate additional tillage before the next date of sowing. The tillage could be done mechanically if separate areas of a field were available, but hand labor would be required if plants on different dates were placed in a single row for each parent. One disadvantage of separate blocks is the time required to walk between different areas of the field to check for flowers and to make the necessary hybridizations. A second disadvantage is that the method cannot be used conveniently for paired-parent arrangements.

The advantages and disadvantages of use of the same row for multiple planting dates are the reverse of those described for use of separate blocks. There are two advantages of subdividing a row into sections in comparison with an inter-

spersed planting. First, it is easier to till and plant a section of row than to make individual plantings at regular intervals within a row. Second, plants from the first date of an interspersed planting may suppress growth of adjacent plants from later dates; this is not a problem when separate sections of row are used. The primary advantage of interspersed planting on different dates is realized in a testcross nursery when open-pollination is used to obtain hybrid seed. Multiple-date plantings are interspersed along the rows of the male parent to obtain a more uniform distribution of pollen than can be realized when distinct sections of row are used for different planting dates.

The interval between planting dates depends on the difference in maturity among the parents and the average environmental conditions that occur at a location, particularly light and temperature. Practical experience is needed to arrive at the appropriate interval.

Day Length. There are two ways in which day length is used to synchronize flowering of parents. One procedure is to artificially shorten or increase the day length of some parents but not others. Shortening the day length can be accomplished by covering plants with a lightproof container. Increasing the day length requires some type of artificial lighting.

A second procedure that is successful for some species is to use environments in which the day length is short or long enough to promote flowering of all parents simultaneously. The natural day length at higher latitudes changes during the summer, which promotes differential flowering among short-day genotypes. Simultaneous flowering may occur, however, if the parents are grown in an off-season field nursery at a lower latitude, where day length is shorter than the critical photoperiod for all genotypes. A similar response may be observed for genotypes of a long-day species by artificially increasing the day length beyond the critical duration.

Temperature. Temperature can be manipulated to synchronize the flowering of parents in some species. The number of growing-degree-days can be altered artificially by maintaining the temperature of some parents higher or lower than that of other parents.

Grafting. The growth factors responsible for initiating flowering can be transferred from one parent to another by grafting. The scion of a parent with delayed flowering may be grafted to a parent with an earlier flowering tendency. The growth factors from the earlier parent pass to the scion and initiate flowering in the later parent sooner than if grafting were not used.

Pruning. Removal of the growing point from a plant frequently causes the initiation of tillering or branching. The onset of flowering on the new vegetative growth may be delayed compared with that of unpruned plants. The delay caused by pruning may be sufficient to synchronize the flowering of parents.

Flower Removal. Some species have immature flower buds that do not fully develop unless older flowers on the plant are removed. The systematic removal of flowers from parents of such species can extend their normal flowering duration, a delay that may be adequate to permit hybridization with a later-flowering genotype.

Plant Population. Flowering on tillers and branches of some species may be delayed compared with flowering on the main stem. Use of a low plant population (density) for some parents can promote the tillering or branching necessary to obtain flowers at the appropriate time for mating with later-flowering parents.

Growth Factors. Application of growth factors to plants is a potential means for artificial induction of flowering. The use of such factors may become more widespread when they have been properly isolated and characterized.

Hybridization

Choice of Female and Male Parents. In a cross between two parents, one must be used as the female and the other as the male. It may make no difference which parent is used as female, or the choice may need to be carefully planned.

One common reason for careful selection of the female parent is to permit the differentiation of progeny that are true hybrids from those that are the result of accidental self-pollinations (Fehr, 1980). To make this distinction, the female parent must have recessive alleles and the male parent dominant alleles for a qualitative character controlled by nuclear genes. Seeds or plants obtained after artificial hybridization are hybrids if they have the dominant character of the male, but are the result of accidental self-pollination if they exhibit the recessive character of the female.

The success of artificial hybridization may be influenced by the parent chosen as female, particularly for interspecific hybridization. Reciprocal crosses often are advisable when matings are attempted between parents of species that may be difficult to cross.

Emasculation of Bisexual Flowers. The elimination of fertile pollen is necessary if bisexual flowers are to be used as the female for artificial hybridization. The pollen in the anthers may be rendered inviable or inactive by appropriate treatments or the anthers may have to be removed manually.

Emasculation Without Anther Removal. Emasculation of the bisexual flowers of some species is unnecessary if the stigma is receptive before the anthers are able to shed pollen. The structure enclosing the reproductive organs is removed, pollen from the male parent immediately is placed on the receptive stigma, and the flower is not covered after pollination. If fertilization does not occur, the

exposed stigma loses its receptivity before the anthers in the flower are able to shed pollen.

Treatment of the anthers with heat, cold, or chemicals has been used to inactivate pollen. Hot-water emasculation consists of soaking flowers at a water temperature high enough to kill the pollen without injuring the stigma. High and low air temperatures have been successfully used to temporarily or permanently inactivate pollen. Soaking flowers in alcohol has been successful for inactivating pollen without injuring the stigma. The proper percentage of alcohol and duration of soaking are important variables for successful alcohol emasculation.

High relative humidity has been used to delay pollen shed without destroying fertile pollen. Although dehiscence control is not a form of emasculation per se, it does permit artificial hybridization of bisexual flowers of some species without anther removal.

Emasculation by Anther Removal. Removal of immature anthers from a bisexual flower is a direct method for eliminating male-fertile pollen. One procedure is to open or remove the structures enclosing the reproductive organs and remove the anthers with a forcep, pencil, or other appropriate instrument. Vacuum pressure sometimes is used to remove the anthers by suction. Removal of the floral structure enclosing the reproductive organs can simultaneously remove the stamens when the two are fused.

Partial removal of anthers by clipping them with a scissors has been used as a rapid method of emasculation. A flower is cut low enough to at least partially remove the anthers without injuring the stigma. With removal of part of the structures that enclose the flower, the cut anthers generally dry up before they can shed pollen. Accidental self-pollination is more of a risk with scissor emasculation than with removal of the entire anther.

Protection of Unisexual Female Flowers. Emasculation is not a concern for flowers that contain only the female organ, that are male-sterile, or that exhibit self-incompatibility. It may be necessary, however, to cover the flowers to prevent undesired pollinations. The same protection may be necessary for flowers that are artificially emasculated but not pollinated immediately after emasculation.

Pollination. Pollination may be done immediately after emasculation or may be delayed for up to several days, depending on the species. Although emasculation and pollination are separate operations, they must be properly synchronized to achieve success. Pollen may be collected and applied manually to the stigma or pollination may be accomplished indirectly.

Direct Pollination. Pollen must be collected from flowers that have not been contaminated by viable pollen from other parents. For some species, the flowers to be used as male are covered for a period of time before pollen is collected, so that any contaminating pollen on the flowers dies.

There are many different methods of pollen collection and application. Entire flowers may be removed and taken to the female parent. Pollen may be collected by placing the male flowers in a bag and tapping the bag to cause pollen shed. Mechanical vibrators may be used to rupture the anthers so that pollen can be collected in a vial. Vacuum pressure may be used to remove undehisced anthers from flowers. Plants may be cut off and placed in a container until pollen is shed naturally and is collected.

Loose pollen may be applied directly on the stigma by pouring it from a container; placing the stigma into the pollen; or applying the pollen with a brush, piece of cotton, or other instrument. An anther or a pollen-laden stigma from the male flower may be manually brushed against the stigma of the female flower to cause pollination.

Indirect Pollination. Pollination can be accomplished without directly applying pollen to the stigma of a female flower. For indirect pollination, the structures enclosing the female organ are completely or partially removed. An inflorescence of flowers shedding pollen can be swirled around the female, causing pollen to fall on the stigma, a method referred to as the go-go or swirl method. The approach method consists of placing an inflorescence of male flowers above the female flowers. Over a period of time, pollen shed from the male falls onto the stigmas of the female parent.

Mutual pollination is the reciprocal pollination of parents, each of which has a high level of self-incompatibility. Inflorescences of the parents are placed together in a bag to allow pollen to pass readily from one to the other and to protect the parents from undesired sources of pollen.

Protection of a Pollinated Flower. A newly pollinated flower may be subject to desiccation and contamination from undesirable pollen. The female organ may be covered by the floral parts that normally enclose it, unless these parts were removed while preparing the flower. Artificial enclosures include such items as bags and soda straws. Flowers of some species require no protection of any kind after pollination.

Labeling of Flowers. Female flowers used for artificial hybridization generally are identified by a tag, wire, bag, or other appropriate label. The label may be placed on the female at the time it is prepared for pollination or after pollination is completed. Information on the label may include the date of female preparation, date of pollination, position of the female flower, name of the female parent, name of the male parent, and name of the person who did the work. The differentiation of female flowers used for hybridization from those that were not used also can be aided by the removal of distinguishing floral parts during hybridization.

Obtaining Seed. The amount of hybrid seed obtained by artificial hybridization varies from one to several hundred per pollination, depending on the species. For some species, inexperienced persons can successfully carry out the work. Successful artificial hybridization for other species requires considerable practice and skill.

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CHAPTER FOURTEEN

Interspecific Hybridization

The genetic improvement of some cultivated plants has involved incorporation of useful genes from other species or genera. New types of plants have been developed and are being contemplated that represent the merger of the chromosome complements of two existing cultivated species. The unique opportunities and challenges presented by interspecific hybridization can be important for plant breeders interested in cultivar development.

Interspecific hybridization refers to crosses between different species, and intergeneric hybridization represents crosses between different genera. Both types of matings are sometimes referred to as wide crosses. For simplicity, interspecific hybridization will be used in the following discussion to include crosses between different species of the same or different genera.

OBJECTIVES OF INTERSPECIFIC HYBRIDIZATION

Improvement of Cultivars of a Species

The level of difficulty in developing a new cultivar generally is related to the number of undesirable characteristics in the parents. The first choice of parental material for most plant breeders is cultivars and experimental lines with desirable quantitative and qualitative characteristics. If genes for a desired characteristic cannot be found in elite parents, a breeder will attempt to find them in a genotype having a minimum number of undesirable traits. Genotypes within the cultivated species generally are evaluated first. If the characteristic cannot be found within the species, the breeder will consider related species within the primary gene pool, then within the secondary gene pool, and finally within the tertiary gene pool (Chap. 11).

Formation of New Species

The formation of a new cultivated species with desirable characteristics from two or more existing species can be a goal of interspecific hybridization. For example, triticale (X *Triticosecale*) is a cultivated species developed from the hybridization of wheat (*Triticum*) and rye (*Secale*). The hexaploid type (2n = 42) has the A and B genomes of wheat and the R genome of rye. An octaploid type (2n = 56) also has been formed, but has less potential for commercial use at present. It has the A, B, and D genomes from wheat and the R genome of rye.

TECHNIQUES FOR GENE TRANSFER

The development of improved cultivars of a cultivated species by the transfer of genes from another species involves two factors: obtaining viable seeds or vegetative propagules in the F_1 and later generations and eliminating undesirable characteristics of the donor species. One or both of these factors may be a consideration in gene transfer between species (Hadley and Openshaw, 1980).

Securing Viable Seeds and Plants

The primary gene pool of a cultivated species includes related taxa with which it can be hybridized to obtain viable hybrid seeds and progeny without the use of special techniques. Because noncultivated types commonly have unusual plant or floral characteristics, making crosses between species in the primary gene pool may be more difficult than crossing genotypes within the cultivated species. Noncultivated types also may have chromosome aberrations that reduce seed set of plants in the F_1 or later generations. In soybean, *Glycine soja* is a member of the primary gene pool of the cultivated species *Glycine max*. *G. soja* × *G. max* crosses generally are more difficult to make than *G. max* × *G. max* matings, due to the small flower size and prostrate growth habit of *G. soja*. Genotypes of *G. soja* may have a chromosome translocation not found in cultivars of *G. max*, which causes partial sterility in the hybrid offspring. Neither factor, however, necessitates special techniques for obtaining viable hybrid seeds or plants.

Gene transfer from species in the secondary and tertiary gene pools may require special techniques to be successful. Failure to obtain viable seeds or plants in the F_1 or later generations can occur at various phases of the transfer program. Various techniques have been used to overcome these barriers (Hadley and Openshaw, 1980).

Lack of Fertilization. The first phase of the gene transfer program that may be unsuccessful is failure of the male and female gametes to unite to form a zygote. Failure of fertilization may be due to lack of pollen germination, insufficient pollen tube growth, or inability of male gametes that reach the embryo sac to

unite with the egg cell. Lack of success at this phase has been referred to as a prefertilization barrier caused by cross-incompatibility. A number of techniques have been used to successfully obtain zygotes from interspecific crosses that initially demonstrated cross-incompatibility.

Parent Selection. Genetic variability for cross-incompatibility may be present for genotypes within a species. The probability of mating genotypes that are compatible is a function of the number of different genotypes of each species that are used as parents.

Reciprocal Crosses. A guideline that has been used when crossing species that differ in chromosome number is to choose as female the one with the greatest number of chromosomes. Although this guideline has been useful, in some cases use of the species with the lower chromosome number as the female has been more successful. Genetic systems related to cross-incompatibility may permit a successful cross in one direction but not the other, regardless of the chromosome number of the parents. As a result, reciprocal crosses are recommended for interspecific matings that have not been attempted or have not been successful.

Modification of Ploidy Levels. When two species of a cross differ in ploidy level, modifying the ploidy of one species to match that of the other may improve the success of obtaining a zygote. Ploidy level can be modified by inducing chromosome doubling or by crossing plants of the same species with different ploidy levels. An example of this latter possibility would be to obtain tetraploids from crosses between diploid and hexaploid plants of species A to use in crosses to tetraploids of species B.

Pollen Mixtures. Cross-incompatibility is associated with proteins of the pistil that interact unfavorably with proteins of the pollen to prevent normal pollen tube germination and growth (Hadley and Openshaw, 1980). This unfavorable reaction has been avoided in certain interspecific matings by mixing pollen from a compatible species with pollen from an incompatible parent.

Modification of the Pistil. Matings may be hampered by the failure of the pollen tube of a short-styled species to attain a length sufficient to reach the ovule of a long-styled species. Use of the short-styled species as female is one alternative, but the mating may not be successful or the reciprocal cross may be more desirable. It may be possible to use the long-styled species as female by cutting back its style before pollen is applied. This technique can only be successful in species, such as maize, in which the shortened pistil remains receptive to pollen.

Chemical Treatment of the Pistil or Pollen. The compounds in the stigma or pollen that prevent pollen tube growth in an incompatible mating have been removed by application of an organic solvent to the stigma or the pollen. Pollen tube growth may be so slow in an interspecific mating that the egg dies or the

flower aborts before the sperm nuclei reach the ovary. Application of growth regulators has been used to promote more rapid pollen tube growth or to increase the length of time the pistil remains viable.

Large-Scale Matings. The frequency of successful pollinations can be extremely low for interspecific matings. Techniques for making a large number of matings have been used to obtain a limited number of seeds. Emasculation has been avoided or minimized by the use of female parents that are self-incompatible or that exhibit genetic or cytoplasmic-genetic male sterility. Rapid emasculation procedures that minimize, but do not eliminate, self-pollination are particularly useful when a genetic marker can be used to differentiate self-pollinated and hybrid offspring. Efficient pollination procedures are helpful to increase the number of matings.

Protoplast Fusion. It may be possible to use protoplast fusion to produce hybrids that cannot be obtained through sexual fertilization. The ability to fuse protoplasts, culture hybrid cells, and regenerate plants is necessary for utilization of this technique.

Lack of Hybrid Seed Development. Successful fertilization in an interspecific mating may not be followed by normal embryo or endosperm development. A number of causes for abnormal seed development have been suggested (Hadley and Openshaw, 1980): (a) Undesirable interactions between genes of the two species may interfere with cell division and differentiation. (b) There may be an unfavorable interaction in zygotic cells between the cytoplasm and nuclear genes. (c) The genetic relationship between embryo, endosperm, and maternal tissue may not be favorable. (d) The number of ovules fertilized may not be adequate to prevent flower and fruit abortion.

Techniques have been developed to overcome the problem of inadequate hybrid seed development. The success of a technique depends on the biological basis of the problem, knowledge of which may be unavailable to the breeder. As a result, each of the following techniques may have to be attempted to overcome the obstacle.

Parent Selection. Undesirable interactions between genes associated with seed development may be avoided by utilizing as parents an array of genotypes of diverse origin from the two species of a mating.

Reciprocal Crosses. An unfavorable interaction between nuclear and cytoplasmic factors in zygotic cells may prevent normal embryo development. The cytoplasms of the species crossed may differ in their capacity to support development of the hybrid embryo. Making reciprocal crosses between a number of

genotypes of each species increases the opportunity for favorable nuclear-cytoplasmic interactions required for hybrid seed formation.

Reciprocal crosses also provide a means of overcoming undesirable interactions among the embryo, endosperm, and maternal tissue. In a cross between species with different genomes and chromosome numbers, the developing embryo has one set of genomes from each species, the endosperm has two sets of genomes from the female and one set from the male, and the seed coat and other maternal tissues have two sets of genomes from the female. The differential contribution of the male parent to the three tissues may result in unacceptable dosage effects for factors related to the coordination of seed development. The number of chromosomes in the endosperm and maternal tissue depends on the species used as the female. Reciprocal crosses between species provide the best opportunity to obtain a favorable relationship between the tissues of developing seed.

Prevention of Flower and Fruit Abortion. The retention of flowers and fruit after fertilization may depend on the number of ovules in an ovary that contain developing seed. The frequency of successful fertilizations from an interspecific mating may be too low to stimulate the processes necessary for ovary development. Loss of the flower or young fruit may occur so early in seed development that rescue of the embryo is not possible. Growth-promoting substances have been applied to flowers to delay abortion at least long enough to obtain an embryo that could be cultured artificially (Hadley and Openshaw, 1980).

A technique used to provide adequate seed set for normal ovary development is to obtain self-pollinated and hybrid seed in the same fruit. This has been accomplished by applying to the stigmas a mixture of pollen from the female and male parents. Another technique used on flowers with multiple stigmas is to pollinate some of the stigmas with self-pollen and others with pollen from the male parent.

Embryo Culture. When a hybrid seed does not develop to maturity, it may be possible to aseptically transfer its embryo to an artificial medium and regenerate plants directly from embryoids or indirectly from callus tissue. Culture conditions that permit successful embryo culture have been identified for several plant species. In barley, embryo culture is used to obtain haploid plants in a cultivar development program that involves interspecific crosses between wild and cultivated barley (Chap. 27).

Inadequate Growth of Hybrid Plants. Hybrid plants obtained from an interspecific cross may lack sufficient vigor to flower and produce mature seeds. The lack of vigor may be caused by unfavorable interactions between genes of the two species within the nucleus or undesirable interactions between nuclear and cytoplasmic factors. Various techniques have been used successfully to overcome this barrier to interspecific hybridization.

Parent Selection. The use of a number of different genotypes as parents from each species provides an opportunity to identify genetic combinations that favor adequate hybrid growth.

Reciprocal Crosses. Lack of adequate hybrid growth due to an unfavorable nuclear-cytoplasmic interaction may be overcome in a reciprocal cross.

Grafting. A weak hybrid plant may be grafted to a normal individual from one of the species used in the cross. The rootstock of the normal individual may provide the growth substances necessary to obtain flowers, mature seeds, or both on the hybrid.

Hybrid Sterility. A frequent difficulty with interspecific hybridization is partial or complete sterility of the hybrid plant. There may be female sterility, male sterility, or both. The most common cause of hybrid sterility is inadequate chromosome pairing during meiosis. When meiosis occurs in an individual with diploid chromosomes for each genome, homologous chromosomes pair during prophase I and one member of each pair moves to separate poles during anaphase I (Chap. 2). After anaphase II, four nuclei are formed that contain a complete haploid set of chromosomes for each genome. If chromosome pairing does not occur, chromosomes line up independently at the equatorial plate. The number of chromosomes that move to each pole during anaphase I can be considered a chance event. If a complete set of chromosomes from each genome is not present in a nuclei after meiosis, the cell may be inviable. Consider an interspecific cross between a diploid species with five chromosomes in genome A and a diploid species with five chromosomes in genome B. The F_1 hybrid would have five A and five B chromosomes. When the chromosomes fail to pair, the nuclei formed by meiosis may have zero to five of the chromosomes of genome A and zero to five of genome B. The probability that a nucleus would have a complete set of each genome is small, the result of which is a high percentage of nonfunctional gametes. The probability that a functional female and male gamete would unite during self-pollination to form a viable seed is equal to the product obtained by multiplying the frequencies of functional male and female gametes. Consequently, little if any seed set would be expected from self-pollination.

A common technique used to overcome sterility caused by lack of chromosome pairing is to induce chromosome doubling in the hybrid. If the number is doubled, each chromosome would have a homologue with which to pair at meiosis, one chromosome of each genome would be present in the haploid nuclei, and functional gametes would result. If cells of a hybrid with two nonhomologous genomes A and B underwent chromosome doubling, the chromosome makeup of doubled cells that entered into meiosis would be AABB, normal chromosome pairing would occur, and viable gametes would be produced.

Inadequate Growth and Fertility of Hybrid Progeny. Unacceptable performance of the progeny of hybrid plants in the F_2 and later generations can impede the transfer of genes between species. Hybrid breakdown occurs when F_1 plants of an interspecific cross are vigorous and fertile but their F_2 progeny lack vigor and may be sterile. Two causes of hybrid breakdown have been suggested (Hadley and Openshaw, 1980): (a) Homozygous dominant alleles at multiple loci may favor plant development in one species, whereas homozygous recessive alleles at the same loci may favor plant development in another species. The F_1 of a cross between the species would be heterozygous and vigorous, but segregation occurring in the F_2 generation would break up the favorable combinations of dominant or recessive alleles. F_2 plants that did not have a dominant allele at each locus or that were not homozygous for all recessive alleles would lack vigor and might exhibit sterility. (b) Small structural differences that do not affect chromosome pairing in the F_1 hybrid may exist in the chromosomes of the two species. Recombination between the chromosome segments during meiosis could lead to the production of chromosome deficiencies or duplications in the gametes of the F_1 hybrid. Hybrid breakdown would occur if the aberrant gametes were viable, but the aberrancies had deleterious effects on the F_2 generation.

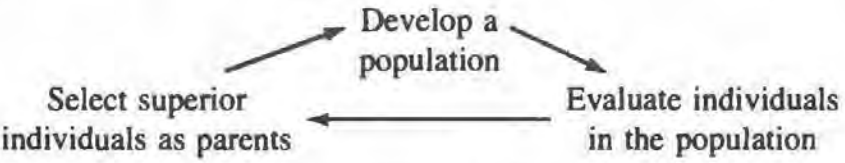
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CHAPTER FIFTEEN

Recurrent Selection

Recurrent selection can be broadly defined as the systematic selection of desirable individuals from a population followed by recombination of the selected individuals to form a new population. The process can be envisioned as a circle that includes population development, evaluation of individuals, and selection of superior individuals as parents to form a new population for the next cycle of selection.



A cycle of selection is completed each time a new population has been formed. The initial population that is developed for a recurrent selection program is referred to as the base population or cycle 0 population. The population formed after one cycle of selection is called the cycle 1 population; the cycle 2 population is developed from the second cycle of selection, and so forth.

The process of recombination, evaluation, and selection occurs routinely in cultivar development programs. For example, the hybridization of elite inbred lines to form single-cross populations, followed by inbreeding, evaluation, and selection of the progeny, could be considered a cycle in a long-term recurrent selection program. However, the term recurrent selection is most often applied to breeding schemes that involve well-defined reference populations and short-term cycles of selection.

The objective of recurrent selection is to improve the performance of populations for one or more characters. The improved populations can be used as a cultivar per se, as parents of a cultivar-cross hybrid, and as a source of superior individuals that can be used as inbred lines, pure-line cultivars, clonal cultivars, or parents of a synthetic.

Successful recurrent selection results in an improved population that is superior to the original population in mean performance and in the performance of the best individuals within it (Fig. 15-1). Ideally, the population will be improved without its genetic variability being reduced so that additional selection and improvement can occur in the future.

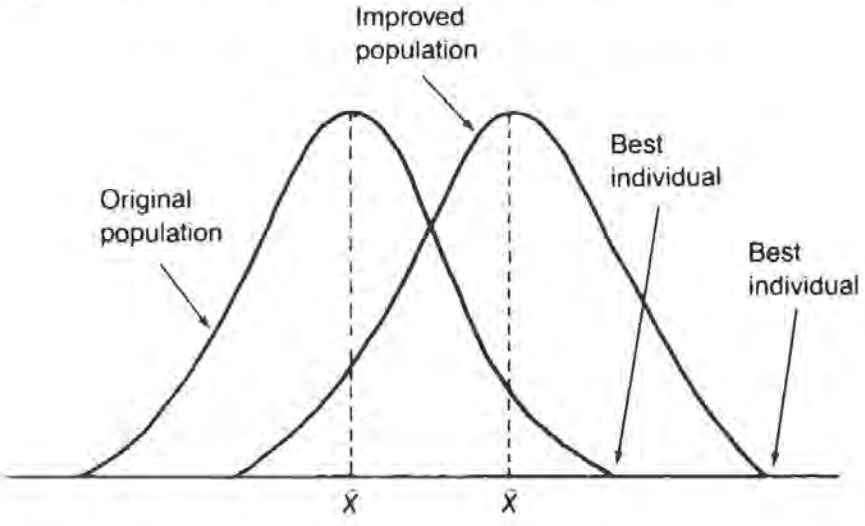
DEVELOPMENT OF BASE POPULATIONS

One consideration in developing a base population is that the parents should have the best performance possible for the characters to be improved through recurrent selection. Another important factor is that the parents should represent an array of different ancestries as a means of maximizing genetic diversity. The two criteria are not always possible to achieve, because potential parents with the best performance may be closely related.

A second consideration is the number of parents that should be used to form the cycle 0 population. The principle is to use as many parents as possible without unnecessarily sacrificing good performance for the characters of interest. The probability of having different alleles present in a population increases with the number of parents and with genetic diversity of the parents. Effective recurrent selection requires a high level of genetic variability in the population for the characters of interest.

A third consideration in the formation of a base population is the number of generations of intermating to conduct in developing the population. Each generation of intermating will improve the opportunity for recombination of genes

Figure 15-1 An idealized example of progress from recurrent selection. The improved population has a higher mean performance than and contains individuals superior to those in the original population. Genetic variability in the improved population has not been reduced by recurrent selection.



from the parents. Additional resources and time are required for each generation of intermating.

Recurrent selection can be conducted for the improvement of a single population (intrapopulation improvement) or for the simultaneous improvement of two populations (interpopulation improvement). The two populations used for interpopulation improvement should display a high level of heterosis for the character of interest when they are crossed together.

EVALUATION OF INDIVIDUALS IN THE POPULATION

Individuals in a population can be evaluated on the basis of their phenotype or on the basis of the performance of their progeny. The methods of intrapopulation improvement can be summarized as follows:

Phenotypic evaluation	*Genotypic evaluation*
Individual plant	Half-sib progeny
Clonal evaluation	Full-sib progeny
	Selfed progeny

Interpopulation improvement involves either half-sib or full-sib progeny evaluation.

Phenotypic evaluation can be based on an individual plant or the vegetatively propagated progeny of the plant in single or replicated plots. The evaluation can be made by visual inspection or by measuring the character of interest.

An individual plant can be evaluated by the performance of its progeny, commonly referred to as a family. The terms half-sib and full-sib refer to the genetic relationship among families, not to the individuals within a family. Half-sib families are formed by crossing a series of individuals to one common parent, which is referred to as the tester. Full-sib families are formed by crossing pairs of plants together. Half-sib families are related because they have a common parent, but full-sib families have no parents in common.

METHODS OF INTRAPOPULATION IMPROVEMENT

Recurrent Phenotypic Selection

Cyclic selection in a cross-pollinated population based on the phenotype of an individual plant has been referred to as mass selection, recurrent phenotypic selection, phenotypic recurrent selection, simple recurrent selection, and directed mass selection. The terms tend to be used interchangeably today; however, some people prefer to differentiate between mass selection and the other terms referring to recurrent selection. They refer to mass selection when female plants are selected after they have been pollinated by selected and unselected males in the

population. They use the term recurrent phenotypic selection when the male and female parents are both controlled because only selected plants are intercrossed to obtain seed for the next cycle of selection. In this chapter, recurrent phenotypic selection will refer to phenotypic selection among individuals, regardless of the parental control involved.

Phenotypic selection of individual plants was the earliest method used to improve cross-pollinated species. In maize, farmers would annually select the most desirable ears in the field and use bulked seed from the selected ears to plant the next crop. Selection was based only on the female plant, because the ear was pollinated by both selected and unselected plants. The independent selection by farmers resulted in an array of open-pollinated cultivars that were genetically different.

Recurrent selection on the basis of individual plants that had been open-pollinated has been used in forages to improve populations. Law and Anderson (1940) conducted five cycles of selection for increased leafiness, number of culms, and basal diameter, and decreased plant height among open-pollinated plants of a big bluestem population. Leaf area of individual plants in the first season of plant growth after establishment increased from 1296 to 10,095 sq cm, number of culms increased from 57 to 148, plant height decreased from 132 to 76 cm, and basal diameter increased significantly.

In 1950, there were reports of recurrent selection in side-oats grama and maize based on individual plant performance, in which only selected individuals were recombined for the next cycle of selection. Harlan (1950) began a selection program in 1943 to increase the uniformity of side-oats grama for 18 different plant types, each selected independently in separate subpopulations. The cycle 0 population consisted of seed lots obtained from locations in Arizona, Kansas, Oklahoma, and Texas. He selected 14 plants of each of the 18 different plant types. The selected plants were transplanted to separate isolation blocks and allowed to open-pollinate. Seed was harvested from each block, and a population of approximately 180 individuals was established from each of the 18 subpopulations. Reselection for type was made in each of the subpopulations to form the cycle 2 populations. The recurrent selection practiced by Harlan involved control of both the male and female parents, because only selected plants were intercrossed to form the new population. This was done because he had noted from previous experience that selection was not effective when only the female parent was controlled. Harlan did not use the words mass selection or recurrent selection in discussing his work.

The research of Sprague and Brimhall (1950) in maize illustrated the marked effect that recurrent selection could have on the improvement of a population. The selection by these investigators was based on the phenotype (oil content) of selfed ears from individual plants. By using selfed seed from selected ears to form the new population, they controlled both the male and female parents. Their method of selection is outlined as follows. The genetic improvement of the population is illustrated in Fig. 15-2.

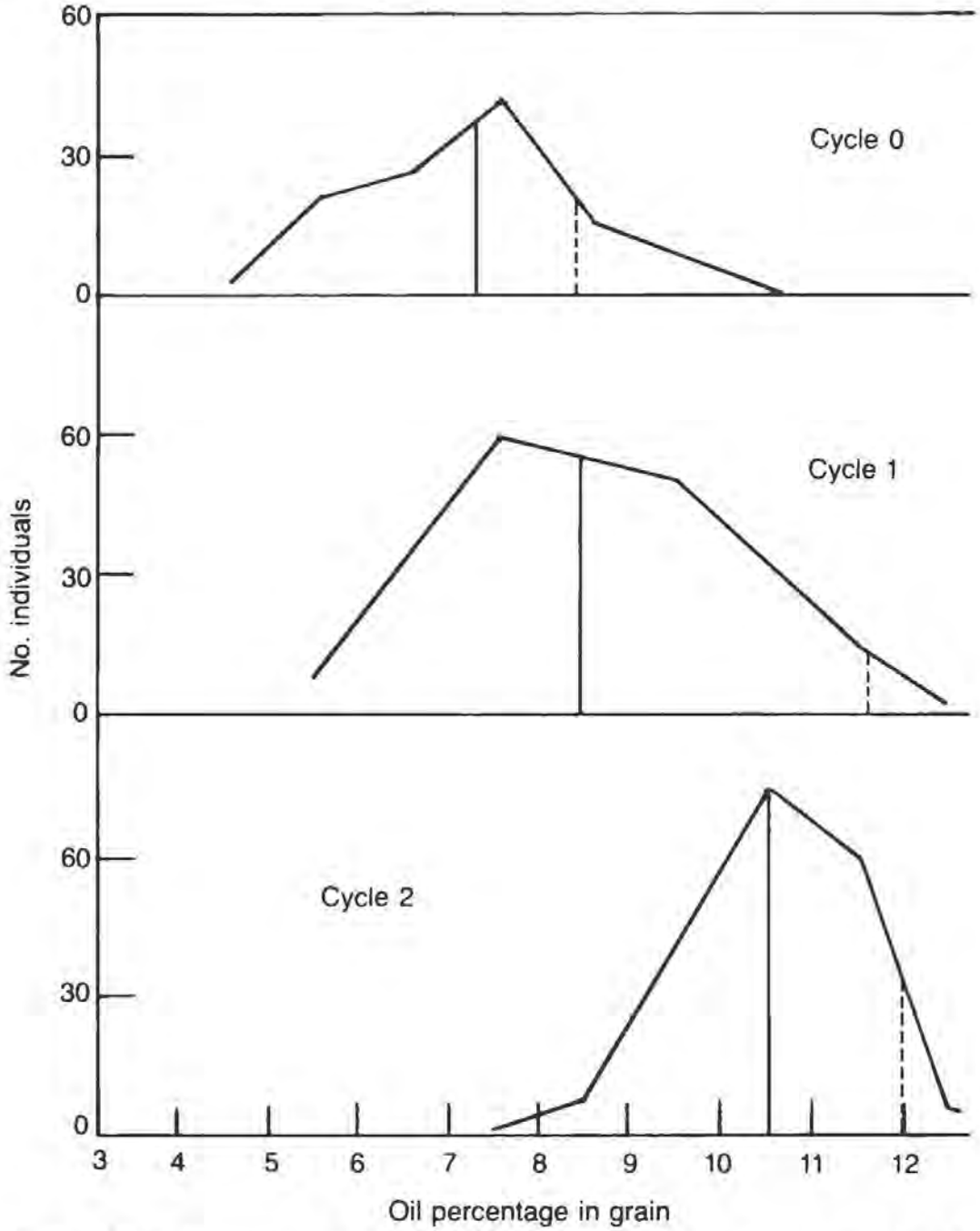


Figure 15-2 Frequency distributions for oil percentage in maize kernels of a population improved by recurrent selection. The solid vertical line in each distribution is the mean of the population, and the dotted vertical line is the mean of the selected individuals. (Adapted from Sprague and Brimhall, 1950.)

Season 1: Individual plants from a population (cycle 0) were self-pollinated. The ears from each plant were harvested separately and seeds from each plant were analyzed for oil percentage.

Season 2: Remnant selfed seeds from each plant selected for oil percentage were planted the following season and all possible intercrosses were made by hand. Equal quantities of seed from each cross were bulked to form the cycle 1 population.

Seasons 3 and 4: Seeds of the cycle 1 population were planted and the procedure used in seasons 1 and 2 was repeated to obtain the cycle 2 population. Subsequent cycles of selection would be conducted in the same manner.

The selection practiced by Harlan (1950) and by Sprague and Brimhall (1950) illustrates techniques for controlling both the female and male parent during recurrent phenotypic selection. Additional examples of selection procedures for control of both parents are provided by Jenkins and colleagues (1954), who selected for disease resistance before flowering in a maize population; by Graham and co-workers (1965), who selected for disease resistance in alfalfa; and by Bennett (1959), who selected for hard seededness in crimson clover.

Jenkins' selection procedure is as follows.

Season 1: The population for selection (cycle 0 population) was planted in the field. During June and July, the plants were inoculated six to eight times with a suspension of *Helminthosporium turcicum* cultures. At pollinating time, the most resistant plants were selected for interpollination. Pollen was collected from the resistant plants, mixed in approximately equal proportions, and placed on the silks of the resistant plants. Seed from the hand-pollinated ears was mixed in equal proportions to form the cycle 1 population for the next cycle of selection.

Season 2: The cycle 1 population was planted in the field and the procedure used in season 1 was repeated to obtain the cycle 2 population. Subsequent cycles of selection would be conducted in the same manner.

Graham's procedure is as follows.

Season 1: About 4900 seeds of a population (cycle 0) were sown in the greenhouse and plants were inoculated in a growth chamber with *Pseudopeziza medicaginis* when 25 to 35 days old. After 4 days in the chamber, plants were moved into the greenhouse. Three weeks after inoculation, disease ratings were made and about 150 plants rated as resistant were selected from the population and reinoculated to eliminate escapes. Approximately 85 plants rated as resistant after reinoculation were selected for intercrossing.

Season 2: The 85 resistant plants were intercrossed to obtain at least 5000 seeds of the cycle 1 population. The time required to complete one cycle of selection (seasons 1 and 2) was about six months.

Seasons 3 and 4: Seeds from the cycle 1 population would be planted and the procedure repeated as described for seasons 1 and 2 to obtain the cycle 2 population. Subsequent cycles of selection would be performed in the same manner.

Bennett's procedure is as follows.

Season 1: One hundred twenty pounds of composited crimson clover seeds from 42 sources (cycle 0 population) were evaluated for hard seed. The seeds were soaked in water for three days. The soaked seeds were rubbed between the hands and broken and swollen seed were floated off with water. The hard seeds were planted in the field, where open-pollination occurred. The seeds were hand threshed and bulked to form the cycle 1 population.

Season 2: One hundred pounds of seeds from the cycle 1 population were soaked for three days. Hard seeds obtained in the same manner as in season 1 were planted in the field. Open-pollinated seed harvested from the planting formed the cycle 2 population. Subsequent cycles of selection would be conducted in the same manner.

The term phenotypic recurrent selection was used by Johnson and El Banna (1957) to describe their selection program in sweetclover. They differentiated between genotypic and phenotypic recurrent selection. Genotypic recurrent selection referred to selection based on the combining ability of an individual, whereas phenotypic recurrent selection was based on the phenotype of the individual. They scored individual plants for growth habit and vigor, and intercrossed only selected individuals for the next cycle of selection. Dudley and colleagues (1963) used the term recurrent phenotypic selection in describing their program of individual plant selection in alfalfa. They used insects and hand pollination to intercross selected individuals.

The expected genetic gain from selection of only the female parent in a recurrent phenotypic selection program is one-half of the amount expected when both parents are selected (Chap. 2 and 17). For that reason, selection before pollination or the intercrossing of selected individuals that are propagated vegetatively or by selfed seed is preferred whenever it does not increase the length of time required to complete a cycle.

One of the problems with phenotypic selection of individual plants is the variability among plants caused by microenvironmental variation, i.e., differences among plants within a field caused by variation in soil type, fertility, moisture, and so forth. When plants are selected for yield, they might all come from one section of the field where the fertility is greatest, even though the individuals are not superior genetically. Gardner (1961) developed a procedure for reducing the effect of microenvironmental variation that involves subdividing a population of plants into blocks (Fig. 15-3, 15-4), sometimes referred to as gridding. His technique is as follows.

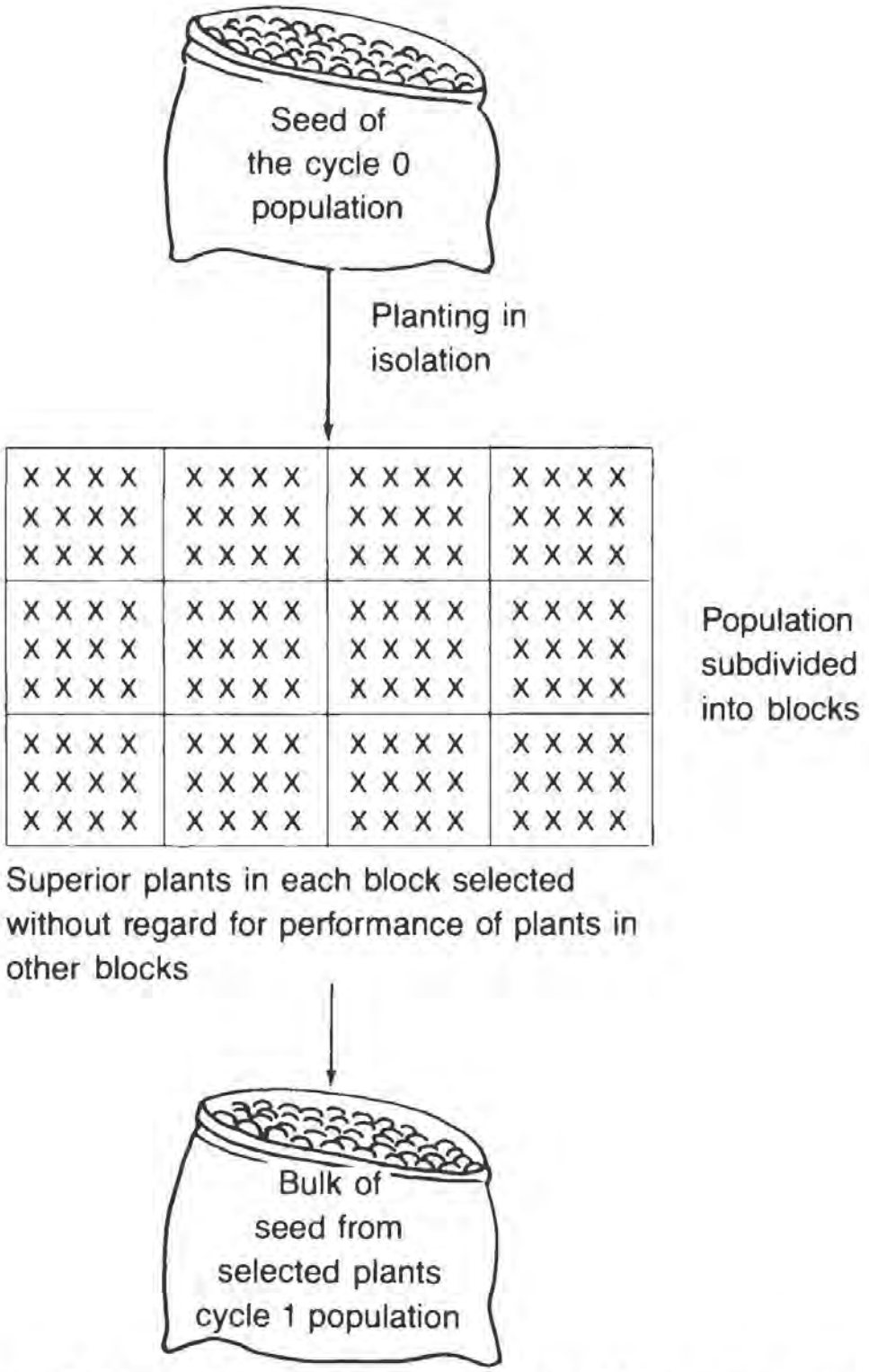


Figure 15-3 The effect of microenvironmental variability on recurrent phenotypic selection can be reduced by subdividing the population (cycle 0) of individual plants into blocks of a grid, selecting the superior individual within each block, and bulking the seed of selected individuals to form the new population (cycle 1) for the next cycle of selection (Gardner, 1961).

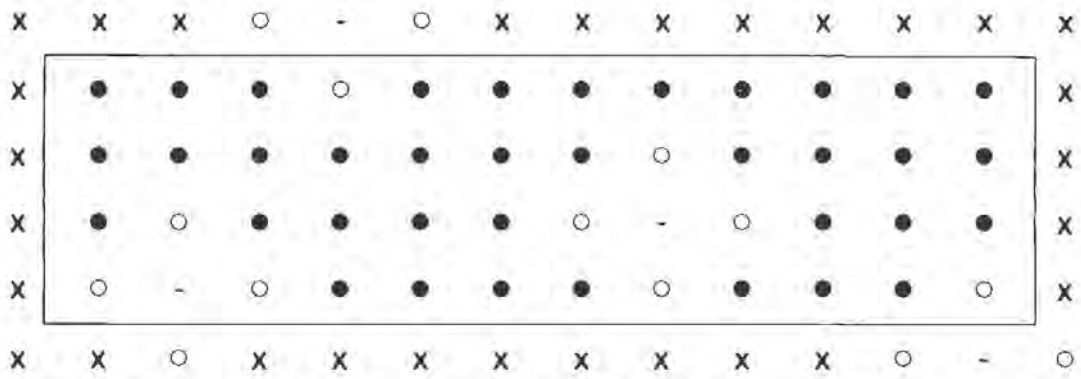


Figure 15-4 One block of a population of individual plants that has been subdivided by gridding to facilitate recurrent phenotypic selection. Only plants ● that have competition on all sides are considered for selection. Missing plants are designated with a − and plants without adequate competition are indicated by o. Plants in adjacent blocks are indicated by x (Gardner, 1961).

Season 1: A nursery of approximately one-fifth hectare is planted with a random-mating population (cycle 0) in an area isolated from other maize. The seeds are planted in hills with 102 cm between rows and 51 cm between hills within the rows (19,370 hills/ha). To obtain uniform competition between plants, two seeds are planted in each hill and the hills are thinned to one plant shortly after plant emergence. If a hill has no plants, remnant seed of the population is planted in the hill and later the hill is thinned to one plant. Special care is taken at planting to place the seed at uniform depth so that seedlings do not emerge sporadically.

The isolation block is irrigated as needed during the growing season to avoid severe drought. All cultural practices (fertilization, cultivation, etc.) in the isolation nursery are done uniformly to avoid unequal treatment of the plants.

Before the time of selection, the field is divided into blocks of 40 competitive plants. A competitive plant is bordered by adjacent plants 51 cm apart within the row and 102 cm between rows. A block is generally four rows (four hills) wide and 10 hills (within the row) long. If the block does not contain 40 competitive plants, it can be extended to 11 or more hills within the four rows. There are 100 blocks of 40 plants within the isolation nursery.

At harvest, the best five to eight plants from each block are visually selected for yield potential. A lodged plant is considered one of the 40 competitive plants in a block. Seeds (ears) from each selected plant are bagged individually and the five to eight bags (plants) from each block are kept together in a larger sack. The seed is dried to a uniform moisture, shelled, and weighed. Twenty-five seeds from the four highest-yielding plants (10% selection intensity) in each block are bulked together (cycle

1 population) to plant the next cycle of selection. Twenty-five seeds from each of four plants in each of 100 blocks results in a 10,000-seed bulk. A similar bulk of 10,000 seeds is saved in case replanting is needed in the next cycle of selection.

Season 2: Seeds from the cycle 1 population are planted in the field and the procedure repeated as in season 1 to obtain the cycle 2 population. Subsequent cycles are conducted in the same manner.

Gardner's concept of stratifying a field for individual plant selection was one of the restrictions used by Burton (1974) in selecting for forage yield of Pensacola bahiagrass. He used the term recurrent restricted phenotypic selection to describe his procedure because he imposed five restrictions on selection among individual plants:

1. The space-planted population was divided into 25-plant square plots in a grid arrangement and the five highest yielding plants in each 25-plant plot were selected, as proposed by Gardner (1961).
2. Only the selected individuals were intercrossed to form the new population.
3. To facilitate intercrossing among selected individuals, two culms with heads ready to flower from each selected phenotype were placed together in water in the laboratory and the collection of flowering heads was agitated each morning to ensure maximum cross-pollination between all selected plants.
4. The use of two heads from each selected phenotype provided equal representation of the parents in the next cycle.
5. The choice of germplasm with a high degree of self-incompatibility reduced the likelihood of selfing of the parents.

Burton's restrictions demonstrate the degree of precision that can be developed for individual plant selection.

Recurrent Half-Sib Selection

Recurrent half-sib selection is a method of intrapopulation improvement that involves the evaluation of individuals through the use of their half-sib progeny. The general procedure for a cycle of selection is to cross the plants being evaluated to a common tester, evaluate the half-sib progeny from each plant, and intercross the selected individuals to form a new population.

There are many alternative procedures for conducting recurrent half-sib selection. The procedures differ by the testers used, the selection of one or both parents, and the seed used for intercrossing. The alternative procedures can be summarized as follows.

Alternative procedures for recurrent half-sib selection.

Tester	Parents Selected	Used for Intercrossing
Population	Female	Half-sib seed
Population	Male and female	Half-sib seed
Population	Male and female	Selfed seed or clones
Outside	Male and female	Selfed seed or clones

An outside tester refers to any germplasm other than the population being improved, such as inbred lines, single crosses, double crosses, and other populations.

The earliest form of recurrent half-sib selection was ear-to-row selection, described by a chemist, C. G. Hopkins, as a method of altering the chemical composition of maize (Hopkins, 1899). His selection work for chemical composition began with the 1896 crop of the open-pollinated maize cultivar 'Burr's White.' It marked the beginning of the long-term Illinois study of selection for seed composition in maize, which has undergone over 70 cycles of selection and is still in progress (Dudley, 1977).

The ear-to-row procedure as described by Hopkins involved the use of the population as the tester, and selection was based on unreplicated tests of the half-sib families in one environment. Because the plants chosen within selected families had been open-pollinated by selected and unselected individuals, only the female parent was selected.

A second procedure for improving open-pollinated maize cultivars by recurrent half-sib selection was proposed by Jenkins (1940). It was based on his observation that the general combining ability of an inbred line could be determined in early generations of inbreeding. The procedure involved the use of the population as the tester, selection of superior half-sib families based on replicated tests, and the use of selfed seed for intercrossing selected individuals.

A third procedure for recurrent half-sib selection was proposed by Hull (1945). The primary difference in Hull's procedure compared with that of Jenkins (1940) is the use of an inbred tester (Fig. 15–7). Hull referred to his procedure as recurrent selection for specific combining ability, because the objective was to develop an improved population or inbred lines from it that could be crossed with the tester to produce commercial seed. The work of Hopkins (1899), Jenkins (1940), and Hull (1945) formed the foundation for the alternative procedures of recurrent half-sib selection.

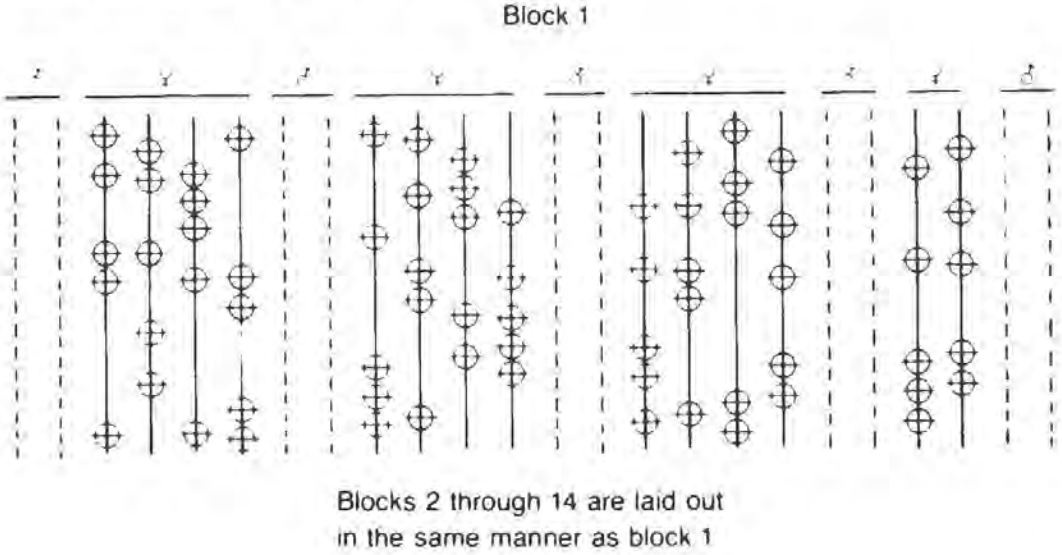
The procedures of recurrent half-sib selection that are available will be reviewed here on the basis of type of tester, parental control, and type of seed used for intercrossing. Alternative strategies within each of the categories will be considered.

Population as Tester, Female Parent Selected, Half-Sib Seed Used for Intercrossing. Ear-to-row selection as described by Hopkins (1899) was based on evaluation of half-sib families in unreplicated plots in one environment. His procedure did not provide information on the relative performance of families under different environmental conditions. To overcome this weakness, Lonnquist (1964) proposed that half-sib families be evaluated at multiple locations and that selection of plants within superior families be conducted in one replication planted in isolation. The procedure is referred to as modified ear-to-row selection because it was developed for use in maize; however, the method can be used in other species in which open-pollinated populations are available. One cycle of selection is completed each season.

Seeds (ears) are harvested from each of 190 plants in a random-mating population (cycle 0). Each of the 190 plants is a separate entry in the yield test of season 1.

Season 1: Six check entries and half-sib seed of the 190 plants are evaluated for yield and other characters. The six checks, consisting of the cycle 0 population and five hybrids, are included each cycle to measure progress from selection. The 196 entries are evaluated in a 14 × 14 triple lattice design with one replication planted at each of three locations. Each plot is a single row eight hills long. Standard techniques for yield evaluation

Figure 15-5 Plot arrangement in one replication of a 14 × 14 triple lattice design for the modified ear-to-row method of recurrent selection for seed yield in maize, as described by Lonnquist (1964). The female rows (half-sib families that are being evaluated for yield) are detasseled. The male rows are a bulk of seed from all the half-sib families in the test. Open-pollinated plants selected within each family are represented by ⊕, each of which is a potential half-sib family for the next cycle of selection.



are used, including planting of excess seed and thinning to the desired stand.

At one location, open-pollinated half-sib seed is obtained for the next cycle of selection (cycle 1 population) by planting the replication in isolation and including male rows for pollination (Fig. 15–5). Each block of the lattice at the location has four plots of the half-sib families alternating with two rows of the male parent. The male parent is a composite of an equal number of seeds from each of the 190 ears (half-sib families). The female rows, including the check entries, are detasseled before pollen shed.

The plots at the two locations that do not involve the special crossing arrangement are evaluated for important characters, and are harvested for yield in the usual way by threshing seed from all plants of a plot in bulk.

At the location where the entries have been detasseled, five plants with the best appearance in each of the 190 rows of half-sib families are marked before harvest by spraying red paint on the tip of their ears. The rows are harvested by hand, the ears from all plants in the row are weighed to determine yield, and the five marked ears are saved for each row.

The data for yield and other characters are summarized for the three locations and the top 20% of the 190 half-sib families (38) are selected. The five marked ears from the 38 selected families constitute the cycle 1 population. Seed from each ear will be a separate half-sib family in the next cycle of selection in season 2.

Season 2: The next cycle of selection is conducted in the manner described for season 1.

Alterations can be made in Lonnquist's procedure. The testing of half-sib families is not restricted to a triple lattice. A randomized complete-block or other experimental design also can be used. The half-sib families can be tested in more than one replication per location and at any number of locations. The number of half-sib families that are evaluated is not limited to any particular number.

Population as Tester, Male and Female Selected, Half-Sib Seed Used for Intercrossing. Selection of both the male and female parents involves the intercrossing of only selected half-sib families (Fig. 15-6). The genetic gain from controlling both parents is twice that expected when only the female parent is selected, but two seasons are required to complete a cycle of selection.

Seeds are harvested from plants in an intermated population (cycle 0). Half-sib progeny from each of the plants will be a separate entry in the replicated tests of season 1. Part of the half-sib seed from each plant is put in storage for potential use in crossing during season 2.

Season 1: The half-sib families are evaluated in replicated tests at several locations, and the superior half-sib families are selected for crossing in season 2.

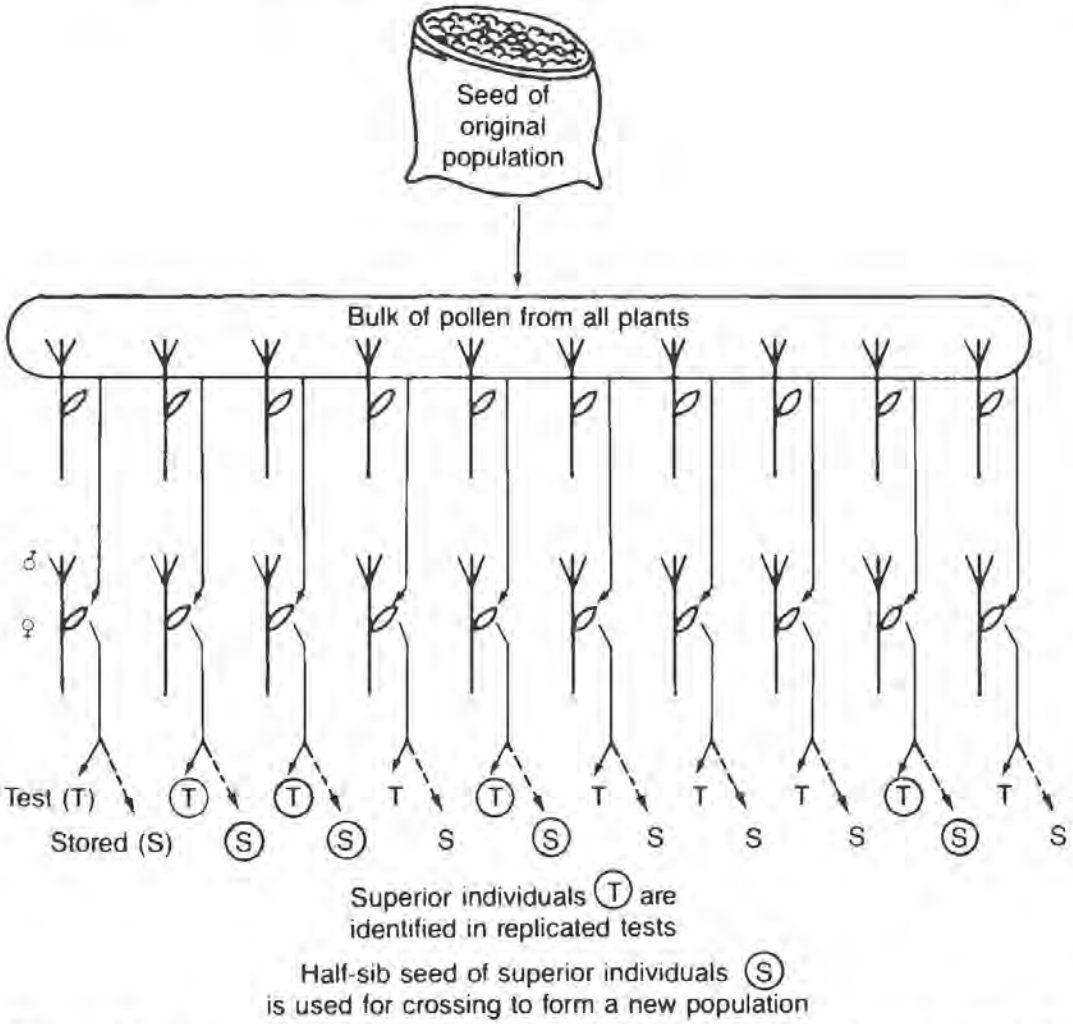


Figure 15-6 Intrapopulation improvement by recurrent selection among half-sib families when the population is used as the tester. Part of the half-sib seed produced on each individual is used for replicated tests and the other part is stored for use in forming the new population.

Season 2: Remnant half-sib seed of selected individuals is used for crossing to form new half-sib families of the cycle 1 population. Part of the half-sib seed produced on each plant is used for replicated tests in season 3 and part is put in storage for potential use in crossing during season 4.

Seasons 3 to 4: The second cycle of selection is conducted by repeating the procedures used in seasons 1 and 2. Subsequent cycles of selection are conducted in the same manner.

Selection can be practiced within half-sib families to increase the genetic gain per cycle. Compton and Comstock (1976) suggested an alteration of Lonnquist's modified ear-to-row selection that permitted selection of both parents and selection within the chosen half-sib families. The procedure requires two seasons per cycle, both of which are suitable for selection of the character of interest.

Seeds are harvested from plants in an intermated population (cycle 0). Half-sib progeny from each of the plants will be a separate entry in the yield test of season 1. Part of the half-sib seed from each plant is put in storage for use in intercrossing during season 2.

Season 1: The half sib families and appropriate checks are evaluated for yield and other characters in replicated tests at several locations. The superior half-sib families are selected for intercrossing in season 2.

Season 2: To permit both selection and intercrossing during season 2, the environment must be one to which the lines are adapted. This prevents the use of some winter nurseries that are suitable for intercrossing but where selection for yield and other important characters is not possible because conditions are not representative of the area in which the lines normally would be grown.

Remnant half-sib seed of the selected half-sib families is taken from storage and a crossing block is planted in isolation. Rows of the half-sib families used as females are alternated with rows of the male parent in an appropriate ratio. The male parent is a composite of an equal number of seeds from each of the selected half-sib families. Standard plot techniques are used to permit visual selection for yield and other characters.

The female rows are detasseled before pollen shed. Within each female row, ears from the five plants that have the best appearance are harvested. The five selected plants from each half-sib family constitute the entries from the cycle 1 population that will be evaluated in the next cycle of selection. Part of the half-sib seed from each plant is used to evaluate the half-sib families in season 3 and part is put in storage to be used for intercrossing selected families in season 4.

Seasons 3 and 4: The second cycle of selection is conducted. Each cycle is conducted in the manner described for seasons 1 and 2.

Population as Tester, Male and Female Parents Selected, Selfed Seed or Clones Used for Intercrossing. The genetic improvement per cycle from half-sib evaluation can be enhanced by the use of selfed seed or clones from selected individuals to form the new population, rather than the use of remnant half-sib seed (Fig. 15–7). The increased gain with the use of selfed seed or clones is due to greater parental control over the alleles that are transferred to the new population (Chap. 17). When half-sib seed is produced, individuals in the population receive half of their alleles from the male parent. Some of the male gametes have favorable alleles for the character under selection, and other gametes have unfavorable alleles. When half-sib seed from selected individuals is used for intercrossing, the unfavorable alleles of the male gametes reduce the amount of genetic improvement in the population. When selfed seed or clones are used to form the new population, only gametes from the selected individuals are passed

to the new population, not the unfavorable male gametes used to produce half-sib seed for testing.

Season 1: Plants from an intermated population (cycle 0) are manually self-pollinated and crossed to the tester. For vegetatively propagated species, half-sib seed is obtained on plants by open-pollination. The selfed seed is stored or the clones are maintained for intercrossing selected individuals in season 3. The half-sib seed is used for testing in season 2.

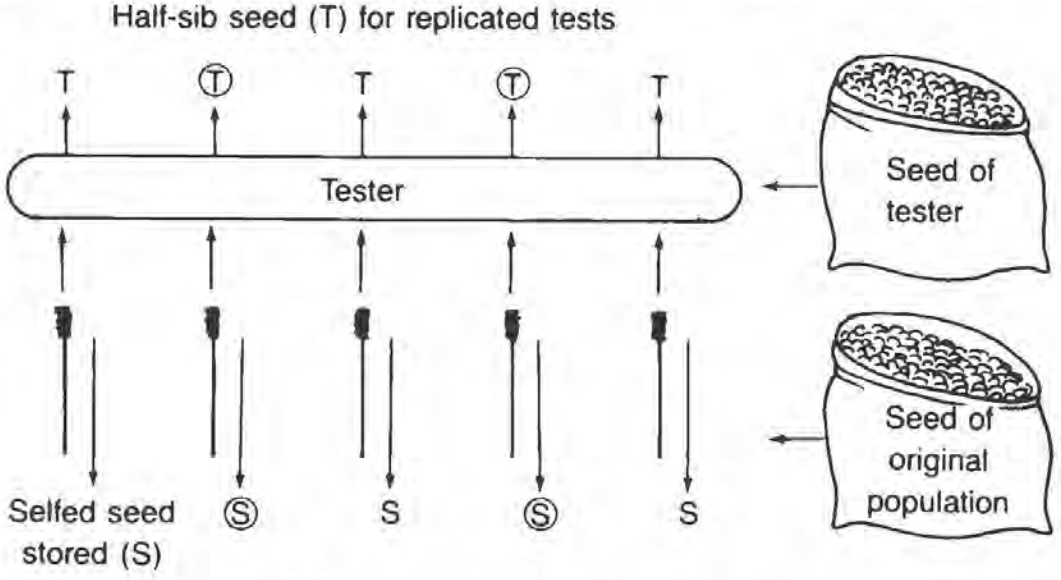
Season 2: The half-sib families are evaluated in replicated tests and the superior ones are selected.

Season 3: Selfed seed or clones from plants that produced selected half-sib families are used for intercrossing to form the cycle 1 population.

Seasons 4 to 6: The second cycle of selection is conducted. The procedure for seasons 1 through 3 is repeated for each cycle of selection.

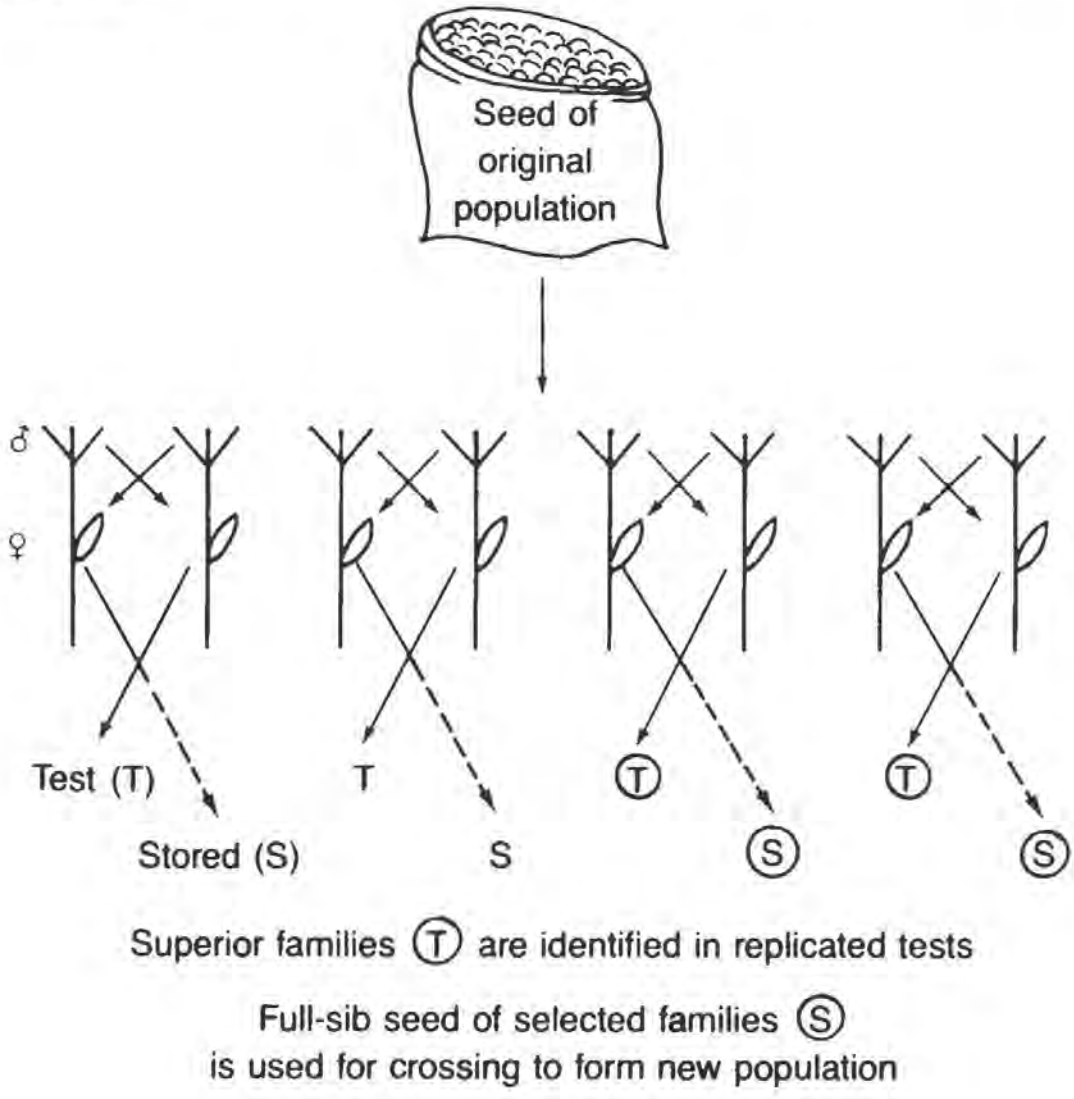
Although the use of selfed seed or clones can increase genetic gain per cycle compared with use of half-sib seed, the genetic gain per year may be less because one extra season is required for recombination. Consequently, the choice between the two procedures will depend in part on the number and types of seasons available to the breeder (Chap. 17).

Figure 15-7 Intrapopulation improvement by recurrent selection among half-sib families when the population is not the tester. Pollen from the test individual is used to pollinate several individuals of the tester to obtain the half-sib seed needed for replicated tests. After superior individuals are identified, selfed seed or vegetative propagules are used for crossing to form the new population.



Tester Other Than the Population, Male and Female Parents Selected, Selfed Seed or Clones Used for Intercrossing. The steps for recurrent half-sib selection with a tester other than the population are the same as those outlined on page 187. The tester used to form the half-sib seed can be a homogeneous inbred line that produces gametes with the same genotype (Fig. 15-7). It also may be a cross or population that provides a heterogeneous array of gametes to the individuals that are to be evaluated. The choice of tester can influence the genetic gain per cycle (Chap. 17). When an inbred tester is used, genetic variability among families may be increased compared with use of the population per se as tester (Sprague and Eberhart, 1977).

Figure 15-8 Intrapopulation improvement by recurrent selection among full-sib families. Part of the full-sib seed produced from paired-plant crosses is used for replicated tests and the other part is stored for use in forming the new population.



RECURRENT FULL-SIB SELECTION

Recurrent full-sib selection is a method of intrapopulation improvement that involves the testing of paired-plant crosses (Fig. 15-8). It is the only method of recurrent selection in which the seeds from two individuals, rather than one, are used for testing and to form the new population.

Season 1: Full-sib families are developed by making crosses between pairs of selected plants in a population (cycle 0). Part of the full-sib seed is put in storage for use in intercrossing selected full-sib families in season 3. The other part of the seed is used for testing in season 2.

Season 2: The full-sib families are evaluated in replicated tests and the superior families are selected.

Season 3: Remnant full-sib seed is used to intercross the selected families. The intercrossed seed that is harvested (cycle 1) is used to begin the next cycle of selection.

Seasons 4 to 6: The second cycle of selection is conducted by repeating the procedures used in seasons 1 to 3. Subsequent cycles of selection are conducted in the same manner.

There are two basic alternatives for the formation of new full-sib families between cycles of selection. One procedure is to cross the selected families and obtain S_0 seed in one season, followed by paired-plant crosses to form full-sib families during the next season as described above. The second procedure is to form new full-sib families in a single season of crossing, thereby reducing the number of seasons per cycle (Hallauer and Miranda, 1981). In the second procedure, full-sib seeds of each of the selected families would be planted in separate rows. Plants from different rows (families) would be paired to obtain the new full-sib families. This modification would permit a cycle of selection in two seasons, but would reduce the amount of recombination between cycles of selection.

RECURRENT SELECTION AMONG SELFED FAMILIES

Recurrent selection among progeny of self-pollinated plants is a method of intrapopulation improvement that is used in both self- and cross-pollinated crops. It involves the testing of lines after one or more generations of selfing followed by intercrossing of individuals to form the new population.

$S_{0:1}$ lines are commonly used for recurrent selection (Fig. 15-9):

Season 1: S_0 plants from an intermated population (cycle 0) are self-pollinated and harvested individually. Part of the S_1 seed from each plant is stored for use in intercrossing selected lines in season 3 and part is used for testing in season 2.

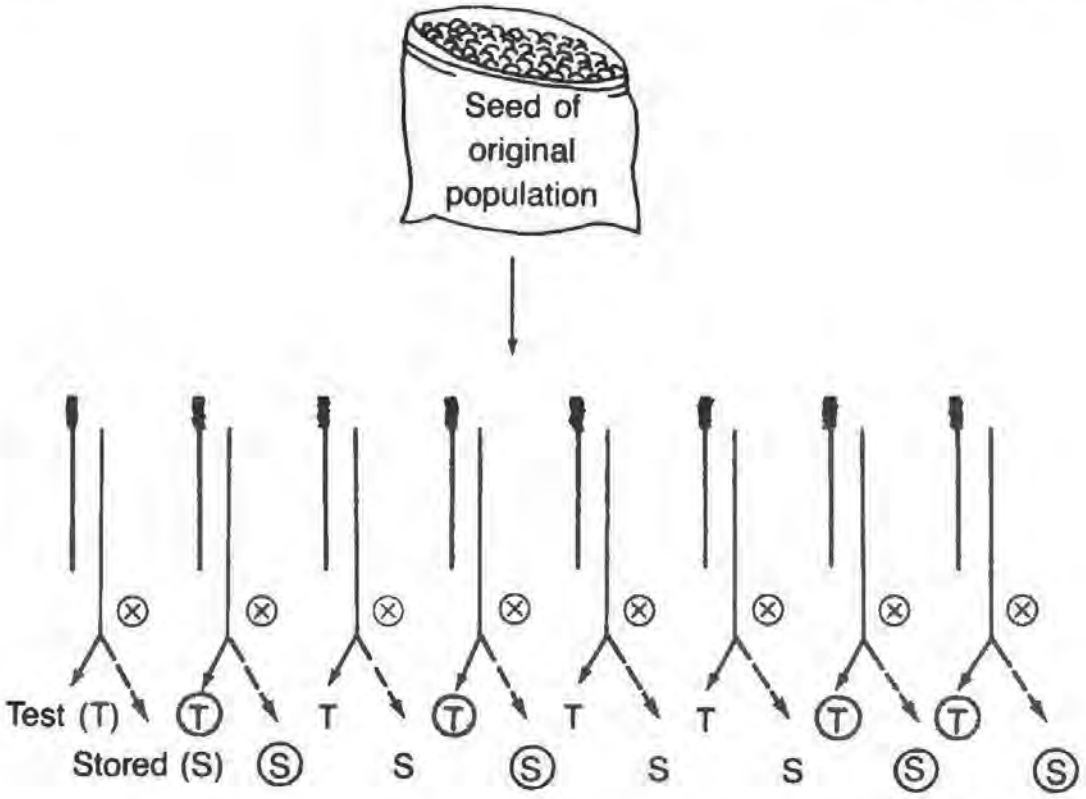


Figure 15-9 Intrapopulation improvement by recurrent selection among $S_{0:1}$ lines. Part of the selfed seed produced on each individual is used for replicated tests and the other part is stored for use in forming the new population.

Season 2: The $S_{0:1}$ lines are evaluated in replicated tests and the superior lines are selected.

Season 3: Remnant S_1 seed is used to intercross the selected lines. The S_0 seed obtained from the crosses represents the cycle 1 population.

Season 4: The next cycle begins. The procedure of seasons 1 to 3 is used for each cycle of selection.

The procedure used to test lines in more advanced generations of self-pollination can be outlined with evaluation of $S_{1:2}$ lines:

Season 1: An intermated population (cycle 0) is planted and selected S_0 plants are self-pollinated manually or naturally. The procedure for seed harvest will depend on the method chosen to maintain the population during selfing (the single-hill procedure of single-seed descent will be used for this example). S_1 seeds are harvested separately from each S_0 plant.

Season 2: Each $S_{0:1}$ line is planted in a separate hill. Plants are self-pollinated within each line, and S_2 seed is harvested from individual plants. Part of the S_2 seed is put in storage for use in intercrossing selected lines during season 4 and the other part is used for testing in season 3.

Season 3: The $S_{1:2}$ lines obtained in season 2 are evaluated in replicated tests and the superior lines are selected for intercrossing.

Season 4: Remnant S_2 seed is used to conduct the first generation of intermating among the lines selected in season 3.

Season 5: Hybrid seed from season 4 is used to conduct the second generation of intermating to obtain S_0 seeds of the cycle 1 population.

Season 6: The next cycle begins by planting the cycle 1 population. Each cycle is conducted by repeating the procedures used in seasons 1 to 5.

There are various ways to modify the procedures just outlined.

1. One season per cycle can be eliminated if lines can be selected and intercrossed the same season. As an example, the schedule for evaluation of $S_{0:1}$ lines would be to self and harvest S_0 plants in season 1 and to test, select, and intercross the $S_{0:1}$ lines in season 2. This can only be done for characters that are evaluated before flowering is completed.
2. The number of seasons per cycle must be increased if insufficient seed is obtained on a single plant to conduct the necessary replicated tests. In the additional season, a progeny row would be grown from each selected plant, a number of plants would be self-pollinated within each row, and bulk seed from the selfed plants in each row would be used for testing the following season. For example, the schedule might be to select S_0 plants in season 1, increase the seed of each $S_{0:1}$ line in season 2, test and select the $S_{0:2}$ lines in season 3, and intercross selected lines in season 4.
3. Sometimes seed from a selfed plant is insufficient for testing and to retain a sample in storage for subsequent intercrossing. In those instances, self-pollinated seed of each line must be increased the same season that replicated tests are being conducted. In a self-pollinated species, seed harvested from the replicated tests can be used if there is minimal outcrossing and the seed is not mixed during harvest. When outcrossing or seed mixtures can occur in the replicated tests, a separate planting is made of each line, plants within each line are self-pollinated, and selfed seed from within each row is bulked for use in intercrossing. When a seed increase is needed, S_2 seed would be used to intercross selected $S_{0:1}$ lines, S_3 seed would be used for $S_{1:2}$ lines, and so forth.
4. The number of seasons per cycle is directly related to the number of intermating generations that are used to develop the population for the next cycle of selection. One or more seasons may be used for intermating.
5. Selection can and should be practiced during any generation in which the lines are grown in a suitable environment. It is common to select among

S_0 plants before and after pollination, and to select among and within rows during subsequent generations of selfing.

METHODS OF INTERPOPULATION IMPROVEMENT

Reciprocal Half-Sib Selection

Reciprocal half-sib selection, also referred to as reciprocal recurrent selection, is a procedure of interpopulation improvement. It was proposed by Comstock and colleagues (1949) as a method for the simultaneous improvement of two populations (Fig. 15-10). Two segregating populations are selected, one of which can be designated A and the other B. Population A is used as the tester to evaluate individuals in population B, and vice versa:

Season 1: One-hundred plants selected in population A (cycle 0) are selfed and crossed to six or more random plants in population B. One-hundred plants selected in population B (cycle 0) are selfed and crossed to six or more random plants in population A. The selfed seed of each plant is put in storage. The half-sib seed is used for testing in season 2.

Season 2: A replicated test is conducted to evaluate the 100 half-sib families of population A and the 100 from population B. On the basis of test results, the top 10 half-sib families are selected from each population.

Season 3: The 10 plants in population A that had superior half-sib progeny performance in season 2 are intercrossed to form a cycle 1 population using the selfed seed produced in season 1. The 10 plants in population B that had superior half-sib progeny performance are intercrossed in the same manner to form a cycle 1 population.

Season 4: The cycle 1 seed of populations A and B are used to conduct the next cycle of selection in the same manner as that described for seasons 1 to 3.

Several modifications of this procedure have been suggested. Russell and Eberhart (1975) suggested a modification based on the consideration that the genetic variance among half-sib families is expected to increase when an inbred tester is used compared with the population as tester. In their procedure, individuals in population A would be selfed and crossed to an inbred line tester from population B that was derived from a previous cycle of selection. Similarly, individuals in population B would be selfed and crossed to an inbred line tester derived from population A. As the program progresses, a superior line from improved population A could become the new tester for population B, and a superior line from population B could become the new tester for population A.

Another modification of reciprocal half-sib selection was suggested by Paterniani (1967) to simplify the production of half-sib seed and to increase its quantity:

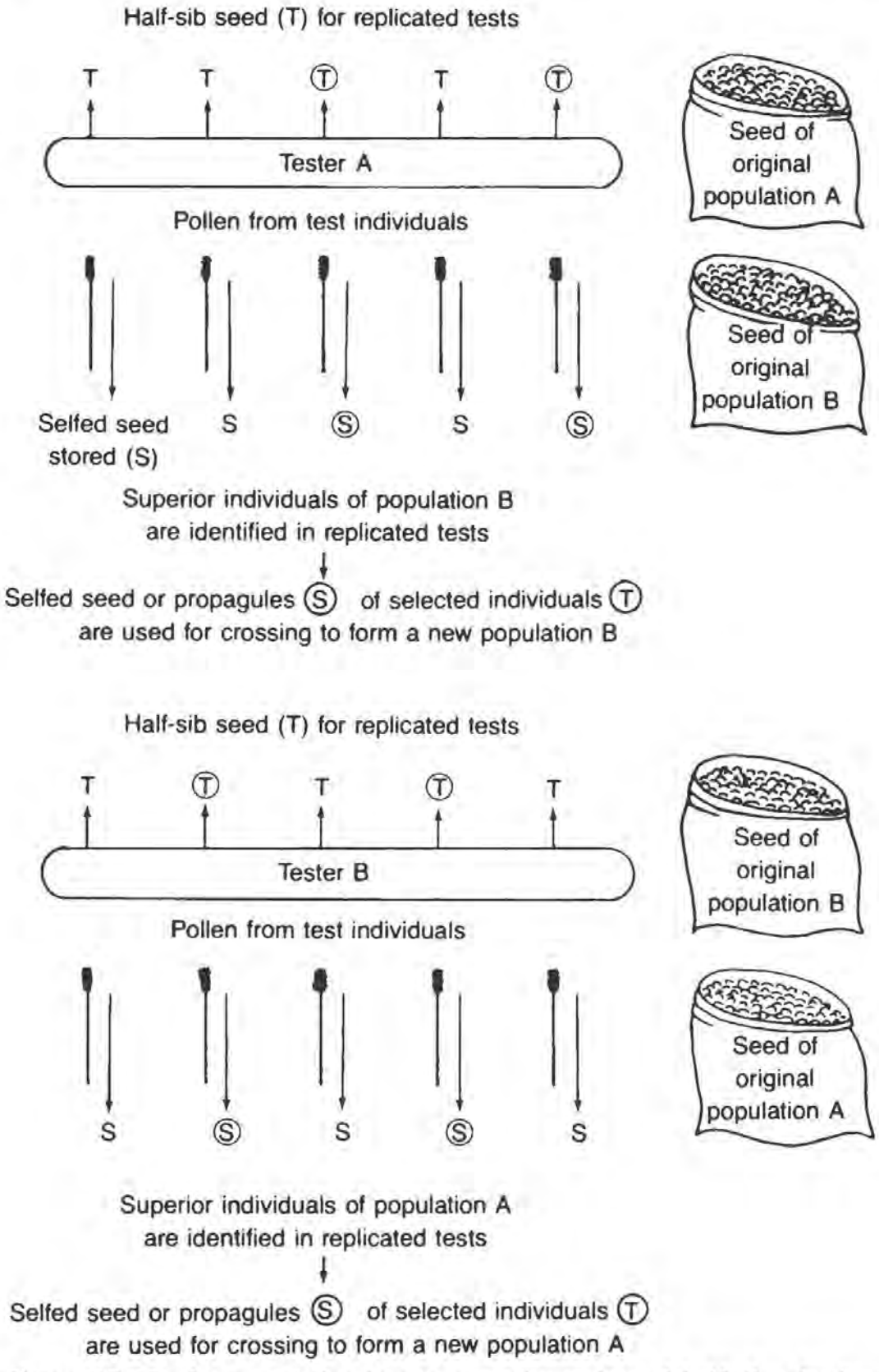


Figure 15-10 Interpopulation improvement by reciprocal half-sib selection. Population A is used as the tester for individuals of population B and vice versa to form half-sib families.

Seeds of population A (cycle 0) and population B (cycle 0) are planted in separate isolations and open-pollination is allowed to take place. Seed is harvested from 100 phenotypically desirable plants in population A and from 100 in population B. Part of the half-sib seed from each plant is retained in storage for use in intercrossing selected individuals in season 3. The other part of the seed is used to produce half-sib seed in season 1.

Season 1: Seed is planted from each of the 100 plants (half-sib families) of population A in separate rows to be used as female and from alternate rows of population B (cycle 0) to be used as male. The ratio of female to male rows will vary with the species. In the female rows, the source of male pollen is removed such as by detasseling in maize. The open-pollinated seed from the female rows is harvested for use in testing during season 2.

The same procedure is used in a separate isolation to obtain open-pollinated seed for the 100 plants (half-sib families) of population B.

Season 2: The seed produced in season 1 is used to evaluate in replicated tests the 100 half-sib families of population A and the 100 of population B. The superior 10 percent of the families in each population are selected.

Season 3: Remnant half-sib seed that had been placed in storage before season 1 is used to intercross the 10 individuals of population A that were found to be superior in season 2. The same is done for selected individuals in population B. The seed of selected lines is planted in isolation for each population, open-pollination is allowed to occur, and seed is harvested from 100 phenotypically desirable plants (cycle 1) to begin the next cycle of selection. Part of the half-sib seed from each plant is put in storage for use in intercrossing of selected individuals and part of the seed is used to produce half-sib seed as in season 1. The procedure of seasons 1 to 3 are repeated for each cycle of selection.

One major effect of Paterniani's procedure on genetic improvement per cycle relates to the use of half-sib seed of superior individuals to form the new population. By use of half-sib seed, the parental control is reduced by 50 percent compared with the use of selfed seed or vegetative propagules from selected individuals. Genetic improvement also is reduced by two seasons of crossing to obtain half-sib seed for testing. When an individual is crossed to the tester the first season, it contributes half of the alleles to the half-sib seed. Its genetic contribution to the half-sib seed used for testing is reduced to one-fourth when its half-sib progeny are crossed a second time to the tester. If each individual contributes such a small fraction of its alleles to the seed for testing, genetic differences among individuals may be minimal due to the masking effect of genes from the tester.

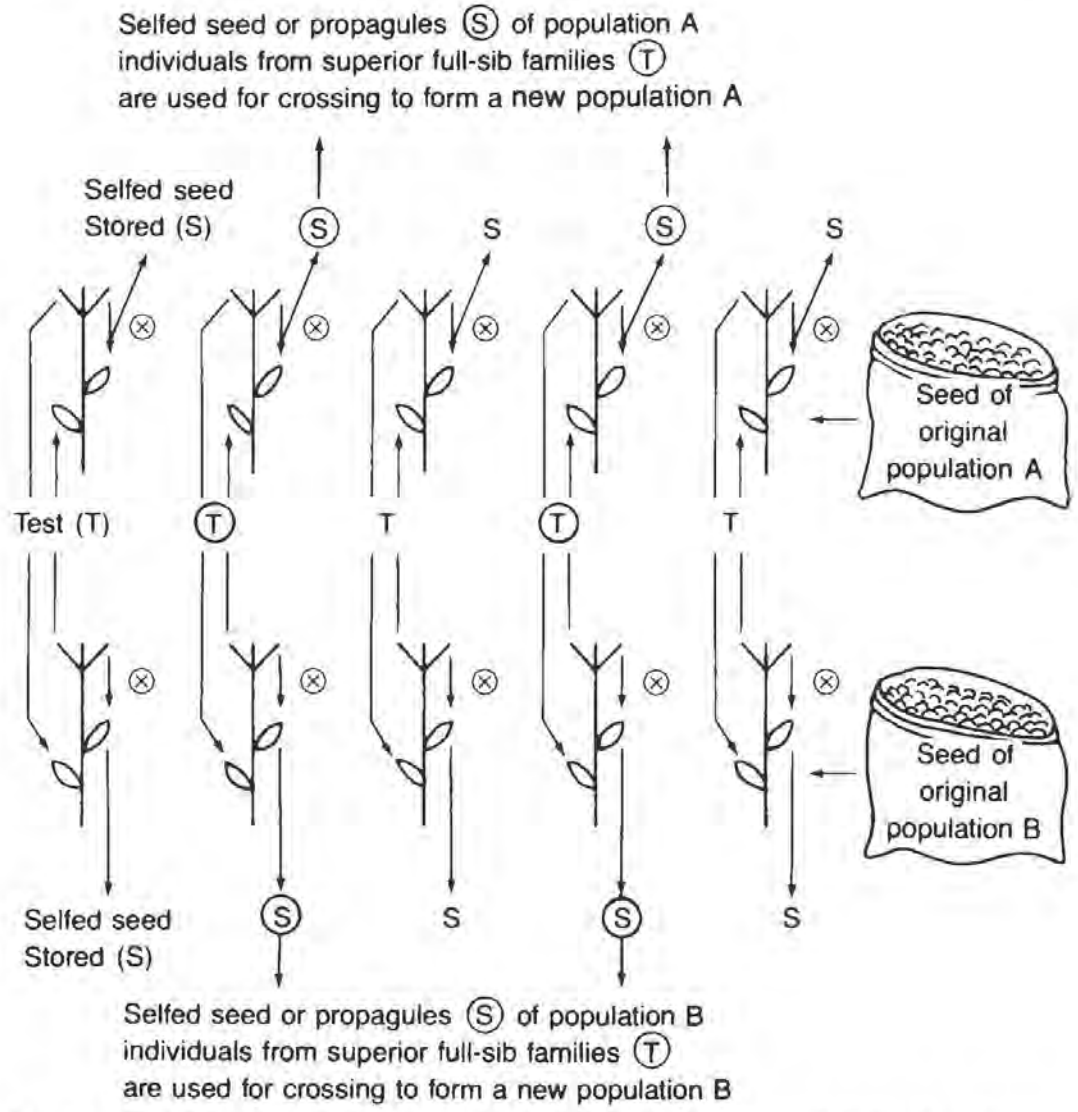


Figure 15-11 Interpopulation improvement by reciprocal full-sib selection. Paired-plant crosses between population A and population B form the full-sib families for evaluation in replicated tests.

Reciprocal Full-Sib Selection

Reciprocal full-sib selection is a method of interpopulation improvement for species in which the commercial product is hybrid seed (Fig. 15-11). It was described independently by maize breeders in Iowa and Nebraska (Hallauer, 1967a,b; Lonnquist and Williams, 1967). A cycle of selection is completed in the fewest number of seasons by the use of plants from which both selfed and hybrid seed can be obtained.

Season 1: Two hundred phenotypically desirable S_0 plants in population A (cycle 0) are paired with 200 plants in population B (cycle 0). For each of the pairs, the plants are selfed and crossed to the other member of the pair. If plants in only one population have the ability to produce both selfed and full-sib seed, the plants in that population must produce all the full-sib seed of the pair needed for testing.

Part of the selfed seed for each plant of a pair is put in storage to be used for intercrossing selected individuals in season 3. The other part of the seed can be used for continued selfing and selection for the development of inbred lines for use in producing commercial hybrids.

Hybrid (full-sib) seed from each pair is used for testing in season 2.

Season 2: The 200 full-sib families are evaluated in replicated tests and the superior 10 percent of the pairs are selected.

Season 3: Selfed seed from storage is used to intercross the 20 individuals of population A that were members of the 20 top full-sib families. Independently, selfed seed from storage is used to intercross the 20 individuals of population B that were members of the 20 top full-sib families. The intercrossed seed of populations A and B represent the cycle 1 populations.

Season 4: Two hundred phenotypically desirable S_0 plants in population A (cycle 1) are paired with 200 plants in population B (cycle 1). The procedure used in seasons 1 to 3 is repeated to obtain the cycle 2 populations. Subsequent cycles are conducted in the same manner.

Reciprocal full-sib selection can be used in species and populations in which selfed and hybrid seed cannot be obtained on the same plant, but the number of seasons per cycle is increased:

Season 1: Two hundred phenotypically desirable S_0 plants in population A (cycle 0) and 200 in population B (cycle 0) are self-pollinated. S_1 seed is harvested from each selected plant. Part of the S_1 seed can be put in storage for use in intercrossing selected individuals in season 4. The other part of the S_1 seed is used for season 2.

Season 2: Each $S_{0:1}$ line of population A is paired with an $S_{0:1}$ line in population B. Crosses are made between the members of each pair to obtain full-sib seed. Bulk pollen from plants in one line can be used to pollinate the other, and vice versa. The full-sib seed from the two members of each pair is bulked for use in testing during season 3.

Season 3: The 200 full-sib families are tested and the top 10 percent of the families are selected.

Season 4: Selfed seed from storage is used to intercross the 20 individuals of population A that were members of the top 20 full-sib families. Independently, selfed seed from storage is used to intercross the 20 individuals of population B that were members of the top 20 full-sib families.

The intercrossed seed of populations A and B represent the cycle 1 populations.

Season 5: Two hundred phenotypically desirable S_0 plants in population A (cycle 1) and 200 in population B (cycle 1) are selfed. The procedure used in seasons 1 to 4 is repeated to obtain the cycle 2 populations. Subsequent cycles are conducted in the same manner.

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CHAPTER SIXTEEN

Genetic Male Sterility for Population Improvement

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

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

DEVELOPMENT OF A POPULATION

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

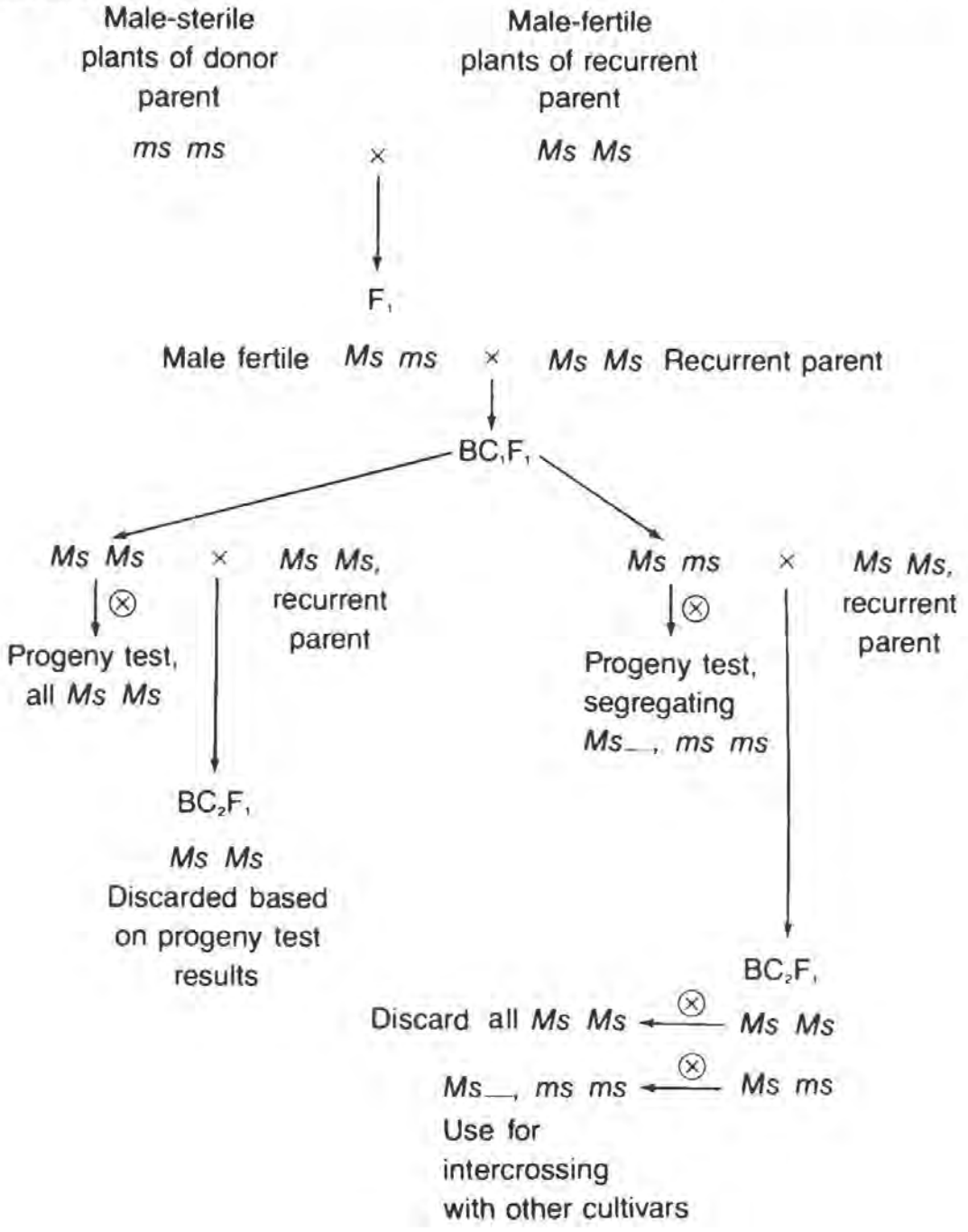
One consideration in developing the population is the percentage of genes from the male-sterile parent, other than the male-sterile (*ms*) allele, that is preferred by the breeder. A percentage less than 50 percent requires backcrossing to the male-fertile parents.

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

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

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

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



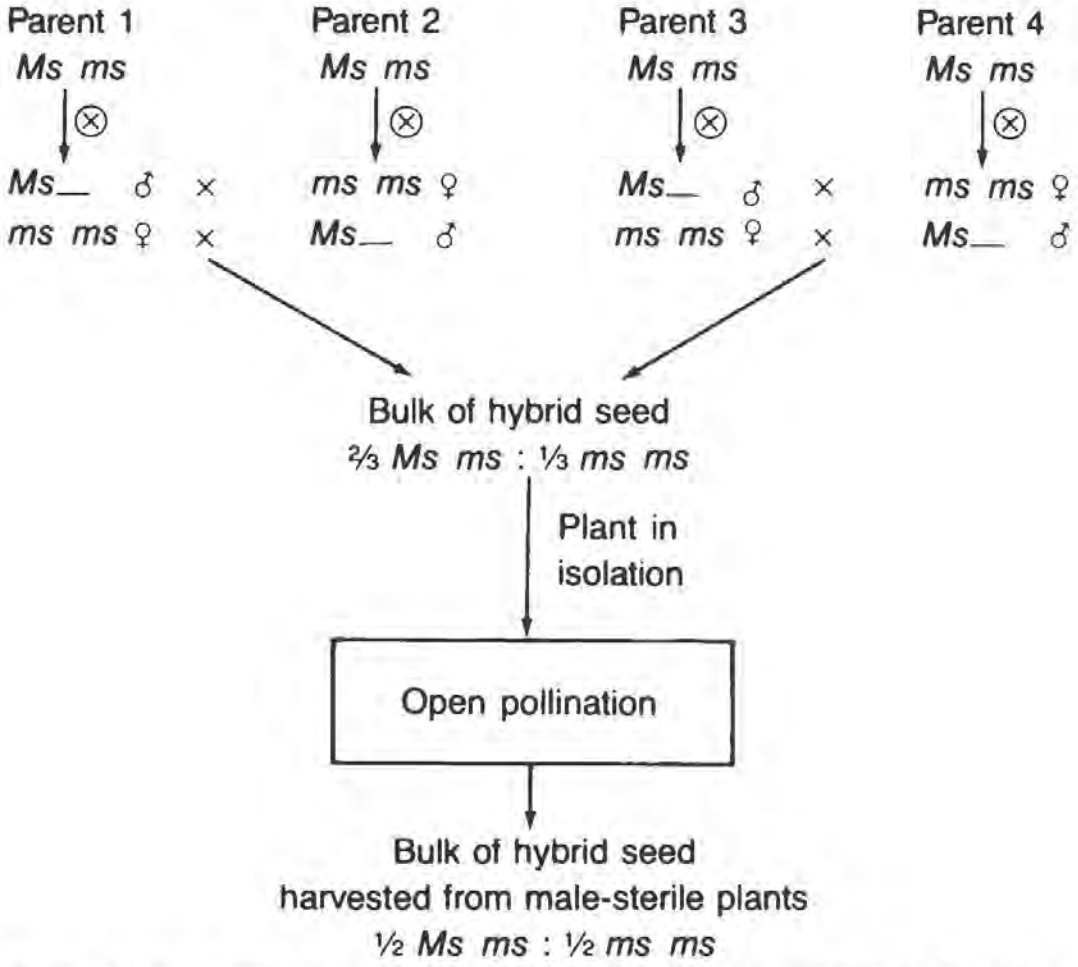


Figure 16-2 Formation of a random-mated population segregating for genetic male sterility by use of four parents heterozygous for the *ms* allele.

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

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

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

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

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

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

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

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

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

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

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

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

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

There is considerable diversity among breeders in the number of backcross

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

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

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

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

UTILIZATION OF THE POPULATION

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

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

Individual Plant Selection

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

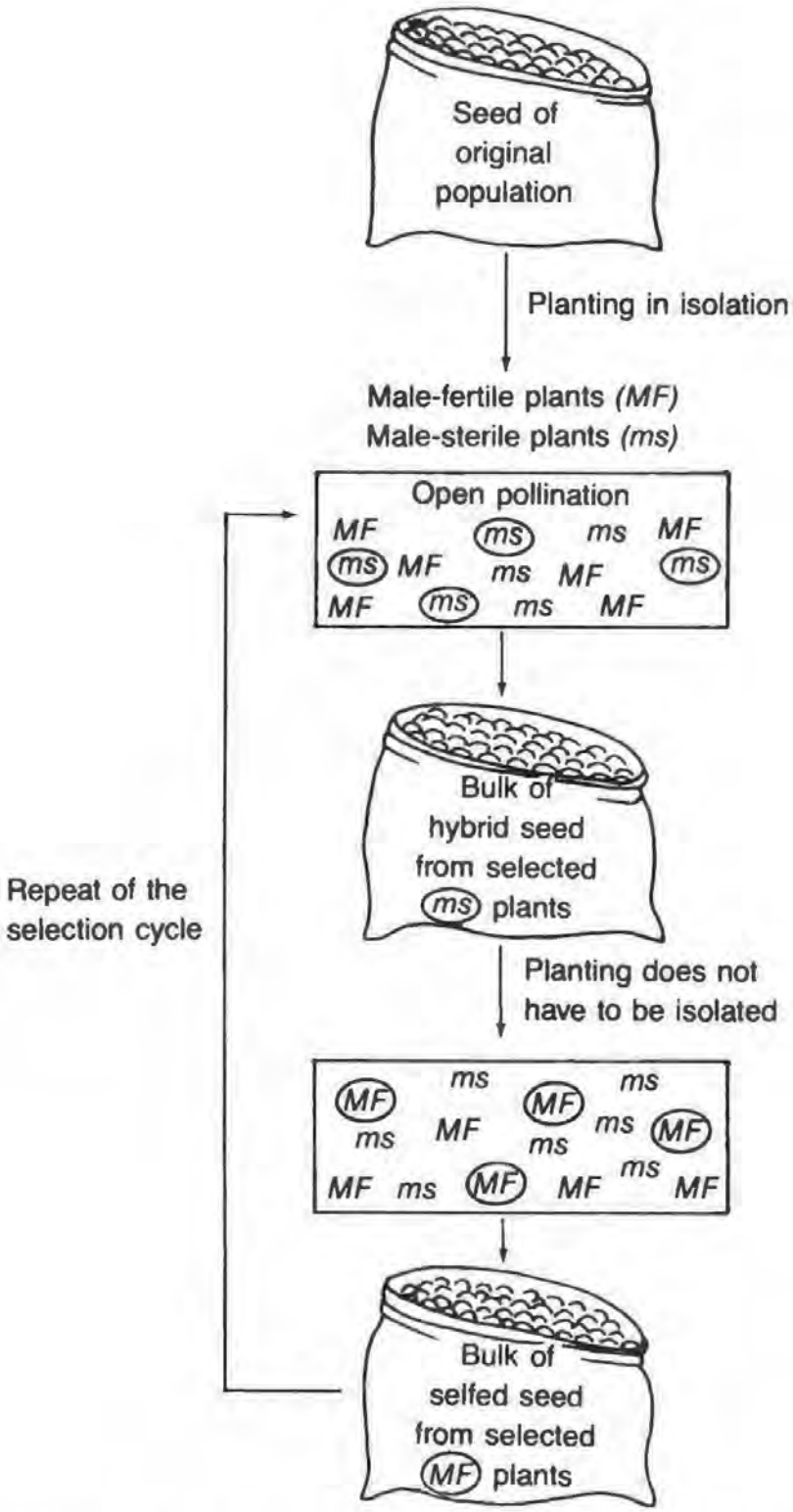


Figure 16-3 Recurrent phenotypic selection among male-sterile and male-fertile plants in alternating seasons, as described by Doggett (1968).

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

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

Selection Among Half-Sib Families

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

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

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

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

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

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

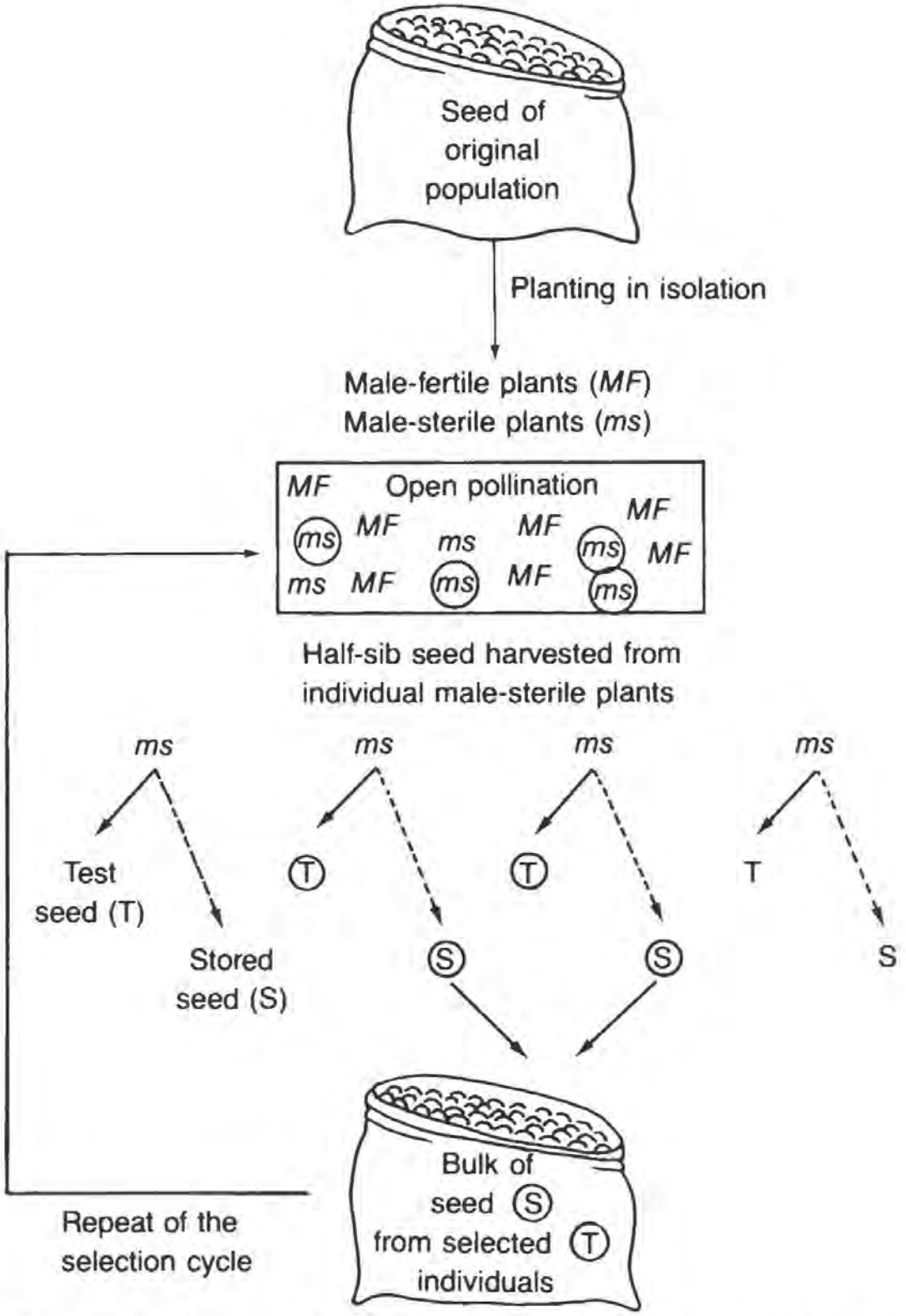


Figure 16-4 Recurrent selection among half-sib families in a population segregating for genetic male sterility.

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

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

Selection Among and Within Half-Sib Families

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

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

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

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

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

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

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

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

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

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

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

Selection Among Plants and Within Half-Sib Families

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

Selection Among Full-Sib Families

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

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

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

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

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

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

Selection Among Selfed Progeny

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

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

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

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

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

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

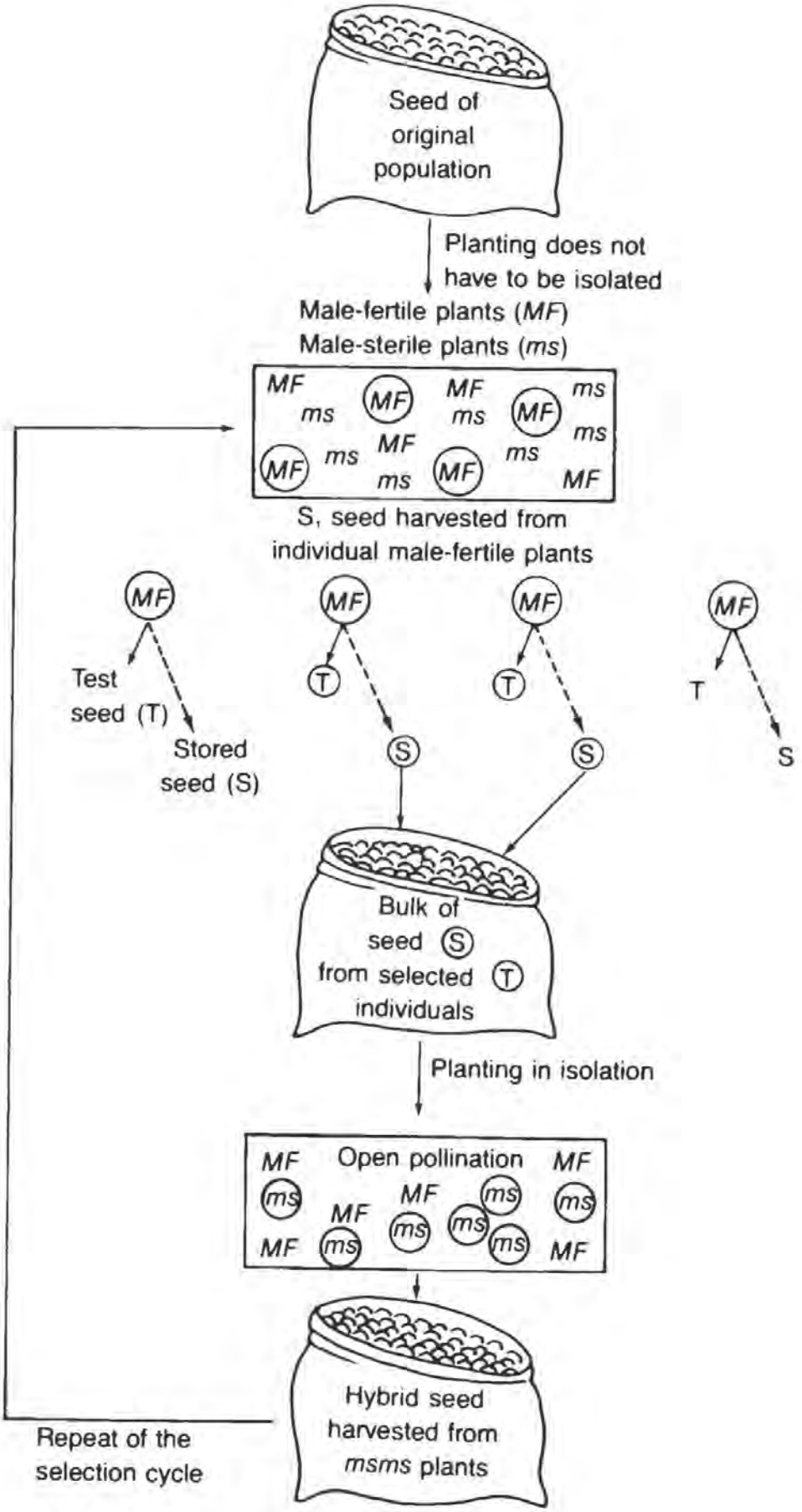


Figure 16-5 Recurrent selection among S_0-derived lines by use of progeny segregating for male fertility and male sterility.

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

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

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

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

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

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

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

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

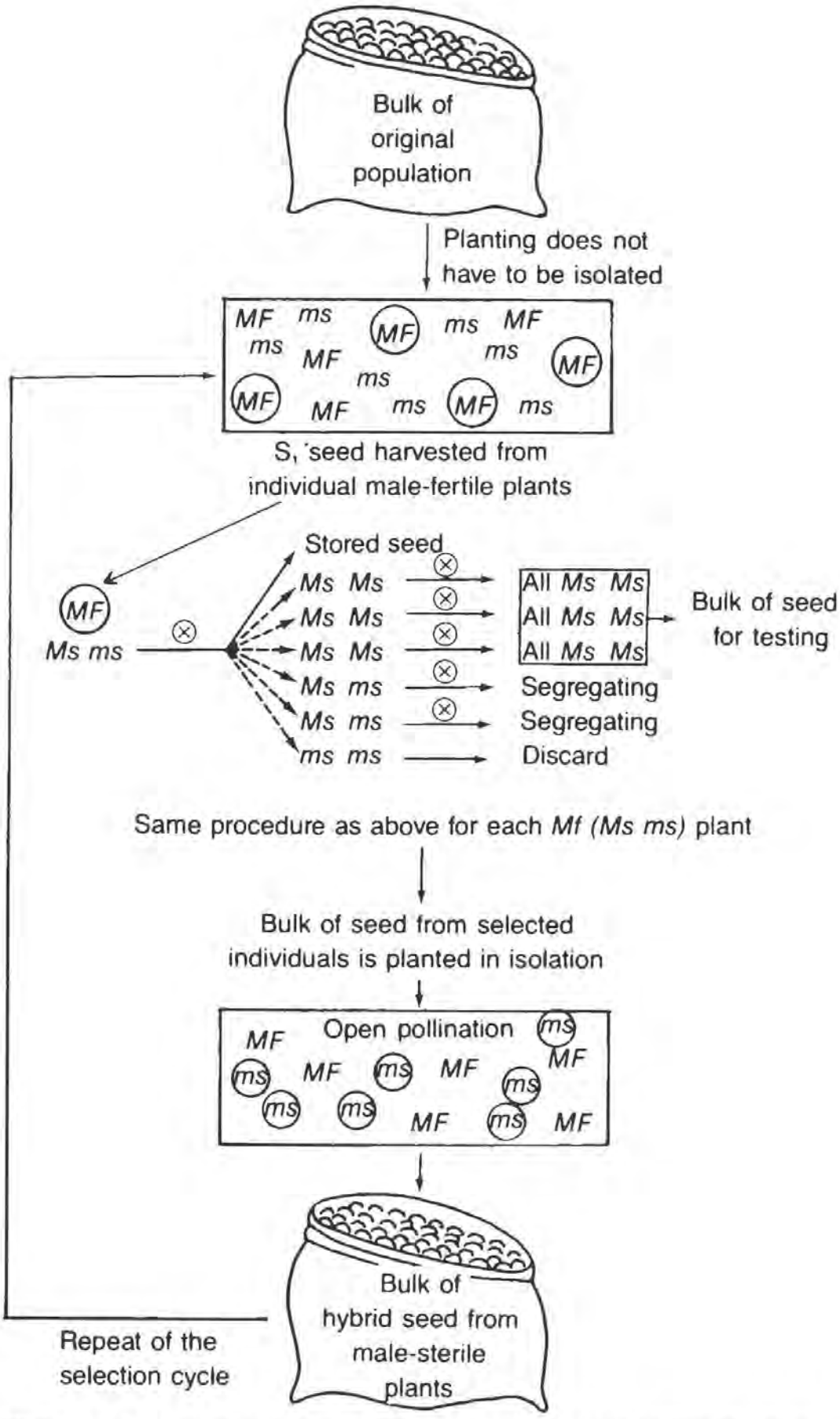


Figure 16-6 Recurrent selection among S_0-derived lines by use of homogeneous male-fertile progeny.

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

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

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

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

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

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

Natural Selection in Populations for Intrapopulation Improvement

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

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

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

INTERPOPULATION IMPROVEMENT

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

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

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

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

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

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

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

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

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

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

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

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

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CHAPTER SEVENTEEN

Maximizing Genetic Improvement

The objective of plant breeding is to improve genetically the performance of cultivars of a species in the most efficient manner possible. Development of an efficient strategy includes selection of an appropriate breeding method and judicious allocation of resources for population development and genotype selection. This requires a comparison of the amount of genetic improvement that can be achieved using alternative breeding methods with the resources that are available.

Plant breeders constantly are trying to identify new procedures that will improve the efficiency of cultivar development. As new ideas are suggested, the breeder must have some criteria for comparing current procedures with the efficiency of genetic improvement using the new system.

The efficiency of alternative breeding strategies can be evaluated as the amount of genetic improvement or gain realized per year. The purpose of this chapter is to review the mathematical equations that are used to compute genetic gain and utilize the equations to illustrate the principles involved in identifying an appropriate breeding strategy.

The concept of genetic gain is based on the change in the mean performance of a population that is realized with each cycle of selection. One cycle includes the establishment of a segregating population, development of genotypes for evaluation, evaluation of the genotypes, selection of the superior genotypes, and utilization of the selected genotypes as parents to form a new population for the next cycle of selection. The length of time required to complete a cycle can vary considerably; therefore, for comparison of alternative strategies, genetic gain is expressed on a yearly basis. The breeder seeks to identify the strategy that utilizes available resources to provide the greatest gain per year.

MATHEMATICAL CONSIDERATIONS

Genetic gain per cycle (G_c) was expressed by Lush (1945) as

$$G_c = h^2D$$

where h^2 is heritability in the narrow sense and D is the selection differential. Genetic gain per year (G_y) is obtained by dividing the genetic gain per cycle by the number of years (y) required to complete a cycle of selection: $G_y = G_c/y$ (Eberhart, 1972).

Heritability is the proportion of the total phenotypic variation expressed among genotypes that can be attributed to genetic differences among them. Narrow-sense heritability is the proportion of total variation attributable to additive genetic variance in the population

$$h^2 = \frac{\sigma_A^2}{\sigma_{ph}^2}$$

where σ_A^2 is the additive genetic variance and σ_{ph}^2 is the phenotypic variance.

The selection differential is the difference between the mean of genotypes selected from a population and the overall mean of the population from which they were selected. If the mean of selected genotypes is 2500 kg/ha and the mean of the population is 2200 kg/ha, the selection differential is 2500 − 2200 = 300 kg/ha. The selection differential can be expressed as

$$D = k\,\sigma_{ph}$$

where k is the selection differential expressed in standard units and σ_{ph} is the square root of the phenotypic variance.

The equation for genetic gain per cycle can be modified by substitutions for h^2 and D:

$$G_c = h^2D = \frac{\sigma_A^2}{\sigma_{ph}^2}\,k\,\sigma_{ph} = \frac{k\,\sigma_A^2}{\sigma_{ph}}$$

Phenotypic Variance

The phenotypic variance includes experimental error (σ_e^2), genotype × environment interaction (σ_{ge}^2), and the genotypic variance (σ_g^2). The square root of the phenotypic variance used in the estimation of genetic gain can be expressed as

$$\sigma_{ph} = \sqrt{\frac{\sigma_e^2}{rt} + \frac{\sigma_{ge}^2}{t} + \sigma_g^2}$$

where r is the number of replications and t is the number of environments in which the genotypes were tested. Genotype refers to an individual plant or its

progeny that is being evaluated. Environment designates locations or years in which tests are conducted.

The experimental error can be subdivided into the variance among plants within a plot (σ_w^2) and the variance from plot to plot (σ^2), expressed as

$$\sigma_e^2 = \frac{\sigma_w^2}{n} + \sigma^2$$

where n is the number of plants within a plot. The variance among plants within a plot includes variation due to environmental effects and genetic differences among plants. Environmental effects include variation in soil fertility, moisture, or any other factors that would cause genetically identical plants to perform differently. Genetic differences among plants in a plot are due to segregation within the progeny of a line or family. The variance within a plot can be subdivided into the environmental variance (σ_u^2) and the genotypic variance (σ_{wg}^2), expressed as

$$\sigma_w^2 = \sigma_u^2 + \sigma_{wg}^2$$

The equation for genetic gain per year can be summarized by substitutions for σ_{ph}.

$$G_s = \frac{k\sigma_A^2}{y\sigma_{ph}}$$

$$G_s = \frac{k\sigma_A^2}{y\sqrt{(\sigma_e^2/rt) + (\sigma_{ge}^2/t) + \sigma_g^2}}$$

$$G_s = \frac{k\sigma_A^2}{y\sqrt{\{[(\sigma_w^2/n) + \sigma^2]/rt\} + (\sigma_{ge}^2/t) + \sigma_g^2}}$$

$$G_s = \frac{k\sigma_A^2}{y\sqrt{(\{[(\sigma_u^2 + \sigma_{wg}^2)/n] + \sigma^2\}/rt) + (\sigma_{ge}^2/t) + \sigma_g^2}}$$

Parental Control

The amount of additive genetic variance is influenced by the parental control exercised in the recombination of selected individuals or families. Parental control in recurrent selection is the relationship between the plant or seed used for identifying superior genotypes (selection unit) and the plant or seed used for recombination (recombination unit).

Parental control is $^1/_2$ when the selection unit is the same as the recombination unit and only the female parent is selected, i.e., when selected female plants are pollinated by both selected and unselected males in the population. Parental

control is $^1/_2$ for recurrent phenotypic selection and modified ear-to-row selection when selection is done after pollination (Table 17-1).

Parental control is 1 when the selection unit is the same as the recombination unit and both parents are selected. Parental control is 1 for recurrent phenotypic selection before pollination, for half-sib family selection when remnant half-sib seed is used for recombination, for full-sib family selection, and for selfed families (Table 17-1).

Parental control is 2 when the selection and recombination units are not the same. Parental control is 2 for half-sib family selection when selfed seed or clones of selected genotypes are used for recombination (Table 17-1). The selection unit is half-sib seed, but the recombination unit is selfed seed or clones

Table 17-1 Methods of Intrapopulation Improvement (Improvement in Population per se)

Method	Seasons per Cycle	Parental Control (c)	σ_g^{2*}		$\sigma_{wg}^{2}\dagger$	
			σ_A^2	σ_D^2	σ_A^2	σ_D^2
Recurrent phenotype selection:						
One parent selected after flowering	1	$\frac{1}{2}$	1	1	0	0
Both parents slected before flowering	1	1	1	1	0	0
Selfed parents (clones) selected, recombined	2	1	1	1	0	0
Half-sib selection:						
Modified ear-to-row						
One parent selected	1	$\frac{1}{2}$	$\frac{1}{4}$	0	$\frac{3}{4}$	1
Both parents selected	2	1	$\frac{1}{4}$	0	$\frac{3}{4}$	1
Population as tester						
Recombination with remnant half-sib seed	2	1	$\frac{1}{4}$	0	$\frac{3}{4}$	1
Recombination with selfed seed (clones)	3	2	$\frac{1}{4}$	0	$\frac{3}{4}$	1
Inbred tester (recombined selfed seed)	3	2	$\frac{1}{4}-\frac{1}{2}$	$0-\frac{1}{4}$	$\frac{3}{4}-\frac{1}{2}$	$1-\frac{3}{4}$
Full sib	2	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$
Selfed progeny:						
$S_{0\text{-}1}$ lines	3	1	1	$\frac{1}{4}$‡	$\frac{1}{2}$	$\frac{1}{2}$‡
$S_{1\text{-}2}$ lines	4	1	$\frac{3}{2}$	$\frac{3}{16}$	$\frac{1}{4}$	$\frac{1}{4}$
$S_{2\text{-}3}$ lines	5	1	$\frac{7}{4}$	$\frac{7}{64}$	$\frac{1}{8}$	$\frac{1}{8}$

*σ_g^2 = Genetic variability among individuals or families.

†σ_{wg}^2 = Genetic variability within families.

‡Coefficients for σ_D^2 that are presented apply when there are two alleles at a locus, each with a frequency of 0.5 (Empig et al., 1972).

Table 17-2 Expected Genetic Gain per Cycle of Selection Under Different Intrapopulation Schemes with Noninbred Parents

Method	Expected Gain per Cycle (G_c)*
Recurrent phenotypic selection:	
Without gridding into subblocks	$\dfrac{k\,c\,\sigma_A^2}{\sqrt{\sigma_u^2 + \sigma^2 + \sigma_{AE}^2 + \sigma_{DE}^2 + \sigma_A^2 + \sigma_D^2}}$
With gridding into subblocks	$\dfrac{k\,c\,\sigma_A^2}{\sqrt{\sigma_u^2 + \sigma_{AE}^2 + \sigma_{DE}^2 + \sigma_A^2 + \sigma_D^2}}$
Modified ear-to-row†	$\dfrac{k\,c\,\frac{1}{4}\sigma_A^2}{\sqrt{\dfrac{\sigma_e^2}{rt} + \dfrac{\frac{1}{4}\sigma_{AE}^2}{t} + \frac{1}{4}\sigma_A^2}}$
Half-sib	$\dfrac{k\,c\,\frac{1}{4}\sigma_A^2}{\sqrt{\dfrac{\sigma_e^2}{rt} + \dfrac{\frac{1}{4}\sigma_{AE}^2}{t} + \frac{1}{4}\sigma_A^2}}$
Full-sib	$\dfrac{k\,c\,\frac{1}{2}\sigma_A^2}{\sqrt{\dfrac{\sigma_e^2}{rt} + \dfrac{(\frac{1}{2}\sigma_{AE}^2 + \frac{1}{4}\sigma_{DE}^2)}{t} + \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_D^2}}$
Selfed progeny, $S_{0:1}$ lines‡	$\dfrac{k\,c\,\sigma_A^{2\prime}}{\sqrt{\dfrac{\sigma_e^2}{rt} + \dfrac{(\sigma_{AE}^{2\prime} + \frac{1}{4}\sigma_{DE}^2)}{t} + \sigma_A^{2\prime} + \frac{1}{4}\sigma_D^2}}$

*σ_u^2 is the within-plot environmental variance, σ_{AE}^2 and σ_{DE}^2 are the additive by environmental and dominance by environmental interactions, σ_A^2 and σ_D^2 are the additive and dominance variance, k is the standardized selection differential, n is the number of plants per plot, r is the number of replications per environment, t is the number of environments.

†If phenotypic selection within rows is practiced, an additional component should be added: $k\,c\,\frac{3}{4}\sigma_A^2/\sqrt{\sigma_u^2 + \frac{3}{4}\sigma_{AE}^2 + \sigma_{DE}^2 + \frac{3}{4}\sigma_A^2 + \sigma_D^2}$.

Source: Adapted from Sprague and Eberhart, 1977.

‡$\sigma_A^{2\prime}$ = additive genetic variance plus a component that is mainly a function of degree of dominance (Empig et al., 1972).

of selected genotypes. The extent of parental control, c, can be incorporated into the numerator of the prediction equation (Table 17-2).

OBTAINING VALUES FOR THE PREDICTION EQUATION

Each of the variables in the prediction equation can be estimated from appropriate experimental studies or can be extrapolated from available data. For certain applications, hypothetical values can be used to compare alternative strategies.

Genetic Variability

The phenotypic variance in the denominator contains the total genetic variance (σ_g^2) expressed among genotypes, including additive, dominance, and epistatic variance (Tables 17-2 and 17-3). The numerator of the prediction equation contains only the additive genetic variance (σ_A^2), because this is the only portion of the genetic variance that is transmitted from the parent to its offspring. Dominance and epistatic variance can be important for the performance of an individual and contribute to the total genetic variance. They are not included in the numerator because intralocus and interlocus interactions from the parent are not transmitted to its offspring (Chap. 6).

The genetic variance (σ_g^2) expressed among genotypes can be readily estimated from an analysis of variance of random genotypes evaluated in multiple environments. Breeders routinely evaluate random lines for quantitative characters, such as yield, in their first replicated test of lines from a population. Estimates of σ_g^2 obtained from evaluation of one breeding method sometimes are extrapolated to obtain estimates for other methods. For example, an estimate of σ_g^2 from an evaluation of half-sib families can be used to estimate σ_g^2 for $S_{0:1}$ or full-sib families. The estimates of σ_g^2 can be biased by the relative importance of additive and dominance variance for the types of families being studied. The variance among half-sib families is additive, whereas the variance among $S_{0:1}$ or full-sib families includes dominance (Table 17-4). The estimate of σ_g^2 equals σ_A^2 from an evaluation of half-sib families and provides a good estimate of σ_A^2 for other types of families. It may not, however, provide a good estimate of σ_g^2 for full-sib and $S_{0:1}$ families if dominance is important. For example, assume

Table 17-3 Expected Genetic Gain per Cycle of Selection for Population Cross Under Different Interpopulation Selection Schemes with Noninbred Parents*

Method	Expected gain (G_s)
Reciprocal half-sib selection†	$\dfrac{k\,c\,\frac{1}{4}\sigma^2_{A(1)}}{\sqrt{(\sigma^2_{e(1)}/rt) + (\frac{1}{4}\sigma^2_{AE(1)}/t) + \frac{1}{4}\sigma^2_{A(1)}}} + \dfrac{k\,c\,\frac{1}{4}\sigma^2_{A(2)}}{\sqrt{(\sigma^2_{e(2)}/rt) + (\frac{1}{4}\sigma^2_{AE(2)}/t) + \frac{1}{4}\sigma^2_{A(2)}}}$
Reciprocal full-sib selection	$\dfrac{k\,c\,\frac{1}{2}\sigma^2_A}{\sqrt{(\sigma^2_e/rt) + [(\frac{1}{2}\sigma^2_{AE}' + \frac{1}{4}\sigma^2_{DE}')/t] + \frac{1}{2}\sigma^2_A' + \frac{1}{4}\sigma^2_D'}}$

*Variance components are defined for the population cross and differ from the corresponding components within a population (Table 17-2) because the gene frequencies in both populations are involved.

†See Table 17-2 for definitions of the symbols in the equations. (1) refers to components in population 1 and (2) to components in population 2.

Source: Adapted from Sprague and Eberhart, 1977.

Table 17-4 Genetic Variability Among Families with Inbreeding (F) When Epistasis Is Negligible ($F = 0$ for F_2 or S_0 Plants)

Half-sib	$\frac{1 + F}{4}\sigma_A^2$
Full-sib	$\frac{1 + F}{2}\sigma_A^2 + \left(\frac{1 + F}{2}\right)^2\sigma_D^2$
Selfed	$(1 + F)\sigma_A^{2\prime} + \frac{1}{4}(1 - F)(1 + F)\sigma_D^2$

*σ_A^2 = additive genetic variance; σ_D^2 = dominance variance; $\sigma_A^{2\prime}$ = additive genetic variance plus a component that is mainly a function of degree of dominance (Empig et al., 1972).

that the true value of σ_A^2 is 40 and σ_D^2 is 10 in a population. If half-sib families are used to estimate σ_g^2, the value obtained would be $\frac{1}{4}\sigma_A = 10$. By doubling the value to estimate σ_g^2 for full-sib families, the value obtained would be 20. However, the true value for σ_g^2 among full-sib families would be $\frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_D^2 = \frac{1}{2}(40) + \frac{1}{4}(10) = 22.5$. The estimated value based on half-sib families (20) would underestimate the true σ_g^2 (22.5) for full-sib families. The possible underestimation or overestimation of variance components should be kept in mind when extrapolating values from one type of family to another. Obtaining estimates of σ_A^2, σ_D^2, and σ_I^2 requires the use of mating designs such as the Design I and Design II that are not commonly used in cultivar development programs (Chap. 6).

The amount of genetic variance expressed among individuals or families in a population is dependent on the amount of inbreeding of the parents (Table 17-4). "Parent" refers to the plant that is selfed (S_1, S_2, S_n), crossed to another plant (full-sib family), or crossed to a tester (half-sib family). F_2 and S_0 plants are assumed to have an inbreeding coefficient of $F = 0$; therefore, the inbreeding among F_2 and S_0-derived lines also is $F = 0$. A description of the derivation of inbreeding coefficients is provided by Falconer (1981).

Selection Intensity (k)

Selection intensity is the percentage of plants or families tested that are selected for recombination. The selection intensity used in the prediction equation is expressed in standard units, k. Derivation of k values is described by Falconer (1981). Values of k increase as the percentage of genotypes selected for recombination decrease (Table 17-5).

Years (y)

The number of years per cycle of selection is the time interval from the evaluation of lines of one cycle until the evaluation of lines in the next cycle. The value

Table 17-5 k Values for Different Selection Intensities

Selection Intensity (%)*	k
1	2.64
2	2.42
5	2.06
10	1.75
15	1.55
20	1.40

*Selection intensity = (number of lines selected/number of lines tested) × 100.

of y includes any delay between the selection of parents and their subsequent mating. If parents are selected in November but are not planted for crossing until May, the number of years per cycle may be increased.

When there is only one season per year during which lines can be evaluated, y will be a whole number. If lines are available for testing in December but cannot be evaluated until May, the early availability of test material in December will not reduce years per cycle. The value of y can only be a fraction when there is more than one season per year available for the evaluation of lines.

Plot-to-Plot (σ^2) and Within-Plot Variance (σ_w^2)

Plot-to-plot variation (σ^2) and within-plot variability (σ_w^2) are determined in sampling experiments in which two or more individual plants are measured in replicated plots. The experimental procedure commonly is referred to as subsampling. Plants can be sampled in all or some of the plots.

The derivation of σ^2 and σ_w^2 can be illustrated with an experiment designed to evaluate seed weight in soybeans. Seed weight (grams per 100 seeds) of 60 $F_{4:5}$ lines of soybeans was measured on three plants in two replications in two environments. The analysis of variance based on samples taken from all plots is presented in Table 17-6. The value for σ_w^2 was 2.20 and for σ^2 was 0.35.

An estimate of σ_w^2 and σ^2 can be obtained by sampling plants for only part of the lines and obtaining a plot mean for all other lines. Two analyses of variance are needed, one for plants within plots and one based on plot means. To compare this procedure with sampling of all plots, single plants for 20 of 60 $F_{4:5}$ lines in the seed weight experiment were used to represent a partial sampling of lines (Table 17-7). The analysis of variance for the 20 lines provided an estimate of σ_w^2 (2.16) that was similar to the value obtained by sampling all lines (2.20). To obtain an estimate of σ^2 with partial subsampling of the lines, an analysis of variance for all 60 lines based on plot means (mean of the three individual

Table 17-6 Analysis of Variance for Seed Weight (g/100 Seeds) of 60 $F_{4:5}$ Lines of Soybeans Tested in Two Replications at Two Environments, with Three Individual Plants Evaluated From All Plots

Source	Degrees of Freedom	Mean Squares		Expected Mean Squares*
Total	719	7.68		
Environments (E)	1	281.28		
Replications/E (R/E)	2	27.45		
Lines (L)	59	59.33	M_1	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2 + nrt\sigma_g^2$
$E \times L$	59	4.00	M_2	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2$
$(R/E) \times L$	118	3.25	M_3	$\sigma_w^2 + n\sigma^2$
Plants/plots	480	2.20	M_4	σ_w^2

*n = plants per plot = 3; r = replications = 2; t = environments = 2.

$\sigma_w^2 = M_4 = 2.20$.

$\sigma^2 = (M_3 - M_4)/n = [(\sigma_w^2 + n\sigma^2) - \sigma_w^2]/n = (3.25 - 2.20)/3 = 0.35$.

$\sigma_e^2 = M_3/n = (\sigma_w^2 + n\sigma^2)/n = 3.25/3 = 1.08$.

$\sigma_{ge}^2 = (M_2 - M_3)/nr = [(\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2) - (\sigma_w^2 + n\sigma^2)]/nr = (4.00 - 3.25)/6 = 0.125$.

$\sigma_g^2 = (M_1 - M_2)/nrt = [(\sigma_w^2 + n\sigma^2 + nrt_{ge}^2) - (\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2) + nrt\,\sigma_g^2)]/nrt = (59.33 - 4.00)/12 = 4.61$.

$\sigma_{ph}^2 = M_1/nrt = (\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2 + nrt\sigma_g^2)/nrt = (\sigma_w^2/nrt) + (\sigma^2/rt) + (\sigma_{ge}^2/t) + \sigma_g^2 = 59.33/(2 \times 3 \times 2) = 4.94$.

Source: Frank, 1980.

Table 17-7 Analysis of Variance for Seed Weight (g/100 Seeds) of Random Sample of 20 of 60 $F_{4:5}$ Lines Analyzed in Table 17-6 (Three Plants Were Evaluated for All Plots of 20 Lines)

Source	Degrees of Freedom	Mean Squares		Expected Mean Squares*
Total	239	5.58		
Environments (E)	1	50.47		
Replications/E (R/E)	2	7.81		
Lines (L)	19	40.08	M_1	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2 + nrt\sigma_g^2$
E × L	19	3.53	M_2	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2$
(R/E)x L	38	2.29	M_3	$\sigma_w^2 + n\sigma^2$
Plants/plot	160	2.16	M_4	σ_w^2

*n = plants per plot = 3; r = replications = 2; t = environments = 2.

Source: Frank, 1980.

Table 17-8 Analysis of Variance for Seed Weight (g/100 Seeds) of 60 $F_{4:5}$ Lines of Soybeans Tested in Two Replications at Two Environments

Source	Degrees of Freedom	Mean Squares		Expected Mean Squares*
Total	239	6.23		
Environments (E)	1	94.50		
Replications/E (R/E)	2	9.37		
Lines (L)	59	19.84	M_1	$\sigma_e^2 + r\sigma_{ge}^2 + rt\sigma_g^2$
$E \times L$	59	1.33	M_2	$\sigma_e^2 + r\sigma_{ge}^2$
$(R/E) \times L$	118	1.08	M_3	σ_e^2

*$\sigma_e^2 = M_3 = 1.08$.

$\sigma_{ge}^2 = (M_2 - M_3)/r = [(\sigma_e^2 + r\sigma_{ge}^2) - \sigma_e^2]/r = (1.33 - 1.08)/2 = 0.125$.

$\sigma_g^2 = (M_1 - M_2)/rt = [(\sigma_e^2 + r\sigma_{ge}^2 + rt\sigma_g^2) - (\sigma_e^2 + r\sigma_{ge}^2)]/rt = (19.84 - 1.33)/4 = 4.63$.

Source: Frank, 1980.

plants per plot) was used to obtain an estimate of σ_e^2 (Table 17-8). The estimate of σ^2 was derived from the relationship $\sigma_e^2 = \left(\frac{\sigma_w^2}{n}\right) + \sigma^2$.

$$\sigma^2 = \sigma_e^2 - \left(\frac{\sigma_w^2}{n}\right) = 1.08 - (2.16/3) = 0.36$$

The value of 0.36 for σ^2 was similar to the 0.35 obtained from sampling plants of all the lines in all of the plots (Table 17-6).

Environmental Variance Among Plants Within Plots (σ_u^2)

The environmental variance among plants in a plot is equivalent to the plant-to-plant variation in a homogeneous inbred line or cultivar. Estimates of σ_u^2 are needed whenever selection is based on a single plant, such as for recurrent phenotypic selection or modified ear-to-row selection that includes selection within rows (Table 17-2).

Genetic Variation Among Plants Within Plots (σ_{wg}^2)

Variation among plants within plots can be due to genetic segregation. The amount of genetic variability within lines for different selection methods is presented in Table 17-1. Effective selection among plants within lines can increase the amount of genetic gain per cycle under appropriate conditions.

Genotype × Environment Interaction (σ_{ge}^2)

The genotype × environment interaction reflects the failure of genotypes to perform the same relative to each other across environments. Change in the rank

among genotypes across environments limits the effectiveness of selection of superior genotypes for recombination and reduces genetic gain per year. Methods of estimating σ^2_{ge} are described in Chap. 18.

COMPARISON OF ALTERNATIVE BREEDING METHODS

The prediction of genetic gain is useful for comparing the potential effectiveness of alternative breeding methods. Recurrent selection in an open-pollinated population of a diploid annual species, such as maize or sunflower, can be conducted by methods including recurrent phenotypic selection, half-sib selection with the population as tester, full-sib selection, and $S_{0:1}$ line selection. The breeder of a self-pollinated species would consider F_2, F_3, or more advanced generations of selfing, unless genetic male sterility was available.

The method with the greatest genetic gain for the character under selection and the resources available can be estimated by computing the predicted genetic gain. The predicted genetic gain for the methods being compared may not be realized, but the relative values for different methods provide a useful estimate of their relative effectiveness. Assume that the predicted genetic gain in yield is 100 kg/ha with $S_{0:1}$ lines and 80 kg/ha with full-sib families. The actual genetic gain realized may be only 50 percent of that predicted for both methods, but the actual gain from $S_{0:1}$ line evaluation would be expected to be greater than from full-sib selection.

There are six steps in the selection of an appropriate breeding method.

Step 1: List the alternatives available for the species being considered. The choices depend on the type of cultivar that will be developed for commercial use and the feasibility of making the required matings. A self-pollinated species that utilizes pure-line cultivars and a species that utilizes hybrid cultivars can both effectively employ $S_{0:1}$ line selection. Methods that involve tests of combining ability, such as half-sib selection with an inbred tester or reciprocal half-sib selection, would not be practical for development of improved pure-line cultivars, but do have potential for development of lines of cross-pollinated species for use in hybrid cultivars.

Step 2: Define the resources available. Resources include the number of seasons that can be utilized each year for population development, inbreeding, family development, and testing. The number of seasons available per year can markedly influence the relative genetic gain per year among methods.

The resources allocated for testing should be kept the same when comparing methods. The number of lines tested and the number of replications and environments used for evaluation are resources that can significantly influence predicted gain per year and that should be kept similar among the methods to be compared.

Step 3: Obtain estimates for the variables in the prediction equation. Methods for obtaining the estimates were discussed previously.

Step 4: Compute predicted genetic gain for the various alternatives. This step will be illustrated with a comparison of seven methods that could be used to improve a population of maize.

The following variance components will be used to compute predicted genetic gain per cycle and per year for yield (q/ha). Assume 14 plants per plot, two replications, and three environments for all replicated tests. The selection intensity will be 10 percent ($k = 1.75$) for all methods.

$\sigma_A^2 = 68$ = additive genetic variance

$\sigma_D^2 = 42$ = dominance variance

$\sigma_{AE}^2 = 70$ = additive × environment interaction

$\sigma_{DE}^2 = 42$ = dominance × environment interaction

$\sigma_e^2 = 96$ = experimental error

$\sigma^2 = 46$ = plot-to-plot environmental variance

$\sigma_w^2 = 700$ = within-plot variance

Predicted genetic gain per year will be computed for four situations.

1. One season per year in which yield evaluation and all breeding operations can be conducted. This situation will be referred to as one season.
2. Two seasons per year can be used for yield evaluation and all other breeding operations. This situation occurs in some tropical areas and will be referred to as two similar seasons.
3. Two seasons per year, one of which can be used for yield evaluation and all other breeding operations and the second of which can be used for all breeding operations, except yield evaluation. Such a situation exists when yield evaluations are possible in a temperate climate, but not in a greenhouse or a winter nursery located in the tropics. The situation will be referred to as two nonsimilar seasons.
4. Three seasons per year, in which the first can be used for yield evaluation and all other breeding operations and the second and third can be used for all breeding operations, except yield evaluation. This situation occurs when greenhouses or winter nurseries are used, and will be referred to as three seasons.

Method 1: Recurrent phenotypic selection with control of only the female parent.

$$G_s = \frac{k\, c\sigma_A^2}{\sqrt{\sigma_w^2 + \sigma_{AE}^2 + \sigma_{DE}^2 + \sigma_A^2 + \sigma_D^2}} =$$

$$\frac{(1.75)(0.5)(68)}{\sqrt{700 + 70 + 42 + 68 + 42}} = 2.0$$

One season, 1 year/cycle

$G_y = G_c/y = 2.0/1 = 2.0$

Two similar seasons, 0.5 year/cycle

$G_y = G_c/y = 2.0/0.5 = 4.0$

Two nonsimilar seasons, 1 year/cycle

$G_y = G_c/y = 2.0/1 = 2.0$

Three seasons, 1 year/cycle

$G_y = G_c/y = 2.0/1 = 2.0$

Method 2: Modified ear-to-row selection with control of only the female parent and no plant selection within rows.

$$G_c = \frac{k\, c\, \frac{1}{4}\, \sigma_A^2}{\sqrt{(\sigma_e^2/rt + (\frac{1}{4}\sigma_{AE}^2/t) + \frac{1}{4}\sigma_A^2}} = \frac{(1.75)(0.5)(0.25)(68)}{\sqrt{[96/(2 \times 3)] + [(0.25)70/3] + (0.25)68}} = 2.4$$

One season, 1 year/cycle

$G_y = G_c/y = 2.4/1 = 2.4$

Two similar seasons, 0.5 year/cycle

$G_y = G_c/y = 2.4/0.5 = 4.8$

Two nonsimilar seasons, 1 year/cycle

$G_y = G_c/y = 2.4/1 = 2.4$

Three seasons, 1 year/cycle

$G_y = G_c/y = 2.4/1 = 2.4$

Method 3: Half-sib selection, population as tester, recombine remnant half-sib seed.

$$G_c = \frac{k\, c\, \frac{1}{4}\, \sigma_A^2}{\sqrt{(\sigma_e^2/rt) + (\frac{1}{4}\sigma_{AE}^2/t) + \frac{1}{4}\sigma_A^2}} = \frac{(1.75)(1)\,(0.25)(68)}{\sqrt{[96/(2 \times 3)] + [(0.25)70/3] + (0.25)68}} = 4.8$$

One season, 2 years/cycle

$G_y = G_c/y = 4.8/2 = 2.4$

Two similar seasons, 1 year/cycle

$G_y = G_c/y = 4.8/1 = 4.8$

Two nonsimilar seasons, 1 year/cycle

Method 4: Half-sib selection, population as tester, recombine selfed seed.

$$G_c = \frac{k\ c\ \frac{1}{4}\ \sigma_A^2}{\sqrt{(\sigma_e^2/rt) + (\frac{1}{4}\sigma_{AE}^2/t) + \frac{1}{4}\sigma_A^2}} =$$

$$\frac{1.75\ (2)\ (0.25)\ 68}{\sqrt{[96/(2 \times 3)] + [(0.25)70/3] + (0.25)68}} = 9.6$$

One season, 3 years/cycle

$G_y = G_c/y = 9.6/3 = 3.2$

Two similar seasons, 1.5 years/cycle

$G_y = G_c/y = 9.6/1.5 = 6.4$

Two nonsimilar seasons, 2 years/cycle

$G_y = G_c/y = 9.6/2 = 4.8$

Three seasons, 1 year/cycle

$G_y = G_c/y = 9.6/1 = 9.6$

Method 5: Full-sib selection.

$$G_c = \frac{k\ c\ \frac{1}{2}\ \sigma_A^2}{\sqrt{(\sigma_e^2/rt) + (\frac{1}{2}\sigma_{AE}^2/t) + (\frac{1}{4}\sigma_{DE}^2/t) + \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_D^2}}$$

$$G_c =$$

$$\frac{1.75\ (1)\ (0.5)\ 68}{\sqrt{[96/(2 \times 3)] + [(0.5)70/3] + [(0.25)42/3] + (0.5)68 + (0.25)42}} = 6.8$$

One season, 2 years/cycle

$G_y = G_c/y = 6.8/2 = 3.4$

Two similar seasons, 1 year/cycle

$G_y = G_c/y = 6.8/1 = 6.8$

Two nonsimilar seasons, 1 year/cycle

$G_y = G_c/y = 6.8/1 = 6.8$

Three seasons, 1 year/cycle

$G_y = G_c/y = 6.8/1 = 6.8$

Method 6: $S_{0:1}$ line evaluation, one recombination between cycles.

$$G_c = \frac{k\ c\ \sigma_A^2}{\sqrt{(\sigma_e^2/rt) + (\sigma_{AE}^2/t) + (\frac{1}{4}\sigma_{DE}^2/t) + \sigma_A^2 + \frac{1}{4}\sigma_D^2}}$$

$$G_c = \frac{1.75\ (1)\ 68}{\sqrt{[96/(2 \times 3)] + 70/3 + [(0.25)42/3] + 68 + (0.25)42}} = 10.8$$

One season, 3 years/cycle

$G_y = G_c/y = 10.8/3 = 3.6$

Two similar seasons, 1.5 years/cycle

$G_y = G_c/y = 10.8/1.5 = 7.2$

Two nonsimilar seasons, 2 years/cycle

$G_y = G_c/y = 10.8/2 = 5.4$

Three seasons, 1 year/cycle

$G_y = G_c/y = 10.8/1 = 10.8$

Method 7: $S_{1:4}$ line evaluation, three recombinations between cycles

$$G_c = \frac{k\,c\,(1 + F)\,\sigma_A^2}{\sqrt{(\sigma_e^2/rt) + [(1 + F)\sigma_{AE}^2/t] + [\frac{1}{4}(1 - F)(1 + F)\sigma_{DE}^2/t] + (1 + F)\sigma_A^2 + \frac{1}{4}(1 - F)(1 + F)\sigma_D^2}}$$

$$Gc = \frac{1.75\,(1)\,(1 + 0.875)\,68}{\sqrt{(96/2 \times 3)}\sqrt{+\,[(1 + 0.875)70/3]}\sqrt{+\,[\frac{1}{4}(1 - 0.875)(1 + 0.875)42/3]}\sqrt{+\,(1 + 0.875)68}\sqrt{+\,\frac{1}{4}(1 - 0.875)(1 + 0.875)42}} = 16.2$$

One season, 8 years/cycle

$G_y = G_c/y = 16.2/8 = 2.0$

Two similar seasons, 4 years/cycle

$G_y = G_c/y = 16.2/4 = 4.0$

Two nonsimilar seasons, 4 years/cycle

$G_y = G_c/y = 16.2/4 = 4.0$

Three seasons, 3 years/cycle

$G_y = G_c/y = 16.2/3 = 5.4$

Step 5: Summarize computed values in a table. The values in Table 17-9 illustrate several important principles concerning the selection of a breeding method and resource allocation.

The method with the greatest gain is not the same for all situations. In our example, $S_{0:1}$ line evaluation gave the greatest gain for one season (3.6), for two similar seasons (7.2), and for three seasons (10.8), but full-sib selection gave the greatest gain for two nonsimilar seasons (6.8).

The value of utilizing more than one season per year varies with the method used and the breeding operations that can be conducted in the additional environments. In our example, one season per year had as much predicted gain as two nonsimilar seasons for recurrent phenotypic selection and modified ear-to-row selection. On the other hand, two nonsimilar seasons provided greater gain than one season for all other methods.

Table 17-9 Predicted Genetic Gain (q/ha) from Selection by Seven Methods

Method*	Gain per Cycle	Gain per Year			
		One Season	Two Similar Seasons	Two Nonsimilar Seasons	Three Seasons
1	2.0	2.0	4.0	2.0	2.0
2	2.4	2.4	4.8	2.4	2.4
3	4.8	2.4	4.8	4.8	4.8
4	9.6	3.2	6.4	4.8	9.6
5	6.8	3.4	6.8	6.8	6.8
6	10.8	3.6	7.2	5.4	10.8
7	16.2	2.0	4.0	4.0	5.4

*1, recurrent phenotypic selection; 2, modified ear-to-row; 3, half-sib, recombine remnant half-sib seed; 4, half-sib, recombine selfed seed; 5, full-sib; 6, $S_{0:1}$ line; 7, $S_{3:4}$ line.

Step 6: Determine the cost per unit of genetic gain. In our example, full-sib selection and $S_{0:1}$ line evaluation gave similar genetic gain when two similar seasons were available (Table 17-9). The cost of conducting full-sib selection includes a yield test and recombination every two seasons. The cost of genetic gain obtained from $S_{0:1}$ line evaluation, however, involves a yield test and recombination every three seasons. The

Table 17-10 Comparison of Time Required for Cultivar Development by Three Methods of Inbreeding in Self-Pollinated Species.

Year	Season	Method of Inbreeding		
		Pedigree	Early-Generation	Single-Seed Descent
1	Summer	F_2 plants selected	F_2 plants selected	F_2 plants grown
	Winter 1*			F_3 plants grown
	Winter 2			F_4 plants grown
2	Summer	$F_{2:3}$ lines selected	$F_{2:3}$ lines selected	F_5 plants selected
3	Summer	$F_{3:4}$ lines selected	Yield test of $F_{2:4}$ lines	$F_{5:6}$ lines selected
	Winter 1		F_5 plants selected	
4	Summer	$F_{4:5}$ lines selected	$F_{5:6}$ lines selected	First yield test of $F_{5:7}$ lines
5	Summer	$F_{5:6}$ lines selected	First yield test of $F_{5:7}$ lines	Second yield test
6	Summer	First yield test of $F_{5:7}$ lines	Second yield test	Third yield test

*The two winter seasons can be used for self-pollination but not for character evaluation.

cost of genetic gain obtained from $S_{0:1}$ line evaluation should be less than for full-sib selection because expensive yield tests are required less frequently.

ENHANCEMENT OF GENETIC GAIN PER YEAR IN PLANT BREEDING

The equations for predicted genetic gain were developed for populations in which some form of recurrent selection is conducted. They also are valuable, however, for comparing the efficiency of selection with conventional breeding methods (Fehr, 1976, 1978). Each of the methods available for developing cultivars has advantages and disadvantages, and the breeder must decide which method is most effective for the resources available. Cultivar development is referred to as a numbers game because the chance of finding a superior cultivar is improved by increasing the number of genotypes that are tested each year. It also can be called a time game because the amount of improvement that can be made over a period of time is influenced by the number of years required for cultivar development. For example, the production of multiple generations per year has become a common practice in the breeding of cultivars of all species. Breeding methods must be adopted that fit well into a multiple-generation system each year (Table 17-10). Pedigree selection was a popular method for inbreeding a population when only one crop was grown each year. It has been replaced in many breeding programs by single-seed descent because of the increased use of greenhouses and winter nurseries, in which visual selection is not possible.

The modern plant breeder must be willing to evaluate new resources that become available and adopt those that increase genetic gain per year at an acceptable cost. In the future, new technology will be developed in plant physiology, molecular genetics, plant pathology, and other disciplines that will aid the breeder. The concepts developed for predicting genetic gain per year in recurrent selection are valuable for evaluating the use of new technology for cultivar development programs. Each of the variables or combinations of variables in the prediction equation can be manipulated.

Years per Cycle

The production of multiple generations of a crop each year has become a fundamental part of modern plant-breeding programs. For breeders in temperate climates, one generation is grown in the field in the area for which new cultivars are being developed. Additional generations, referred to as off-season generations, are grown during the remaining months of the year in a greenhouse or growth chamber, at locations of lower latitude in the same hemisphere, or at a location in the opposite hemisphere where the crop is being grown commercially. Such off-season environments may be used for hybridization, inbreeding, and

seed increase. For some traits they also can be used for evaluation and selection. The extent to which these operations can be conducted depends on the facilities that are available, the crop that is grown, and the character that is under selection. The choice of an appropriate off-season environment is an important decision, particularly for breeders in temperate climates who must choose from the various options that are available. Each of the options have positive and negative aspects that must be considered in making the choice.

Greenhouses and Growth Chambers. Greenhouses and growth chambers are particularly well suited to crops that require limited space for each plant and to breeding operations that require relatively few plants to accomplish. Hybridization is the most common breeding operation conducted in a greenhouse. Hybrid plants also may be grown and generations may be advanced, primarily by single-seed descent. The controlled conditions are regularly used for the evaluation of pest resistance. Seed increase generally is not possible in the limited space available.

A greenhouse or growth chamber has several advantages as an off-season environment. The plants are exposed to less environmental fluctuation and fewer production hazards than occur in the field. The control of environmental conditions can be especially desirable for hybridization and screening for pest resistance. When a greenhouse is at the location where a breeder is stationed, the time, expense, and risk of transporting plant material from one location to another are eliminated. Legal restrictions on the movement of plant material are avoided. There is no possibility that pests will be accidentally moved from one location to another. The growing plants can be observed and manipulated by the breeder and staff without any travel.

Greenhouses and growth chambers have several disadvantages that limit their desirability as an off-season environment. The cost of building and maintaining the facilities can be substantial. Space is limited. Large breeding programs for certain crops require more than a hectare to grow all of their genetic material. The cost of building and operating a greenhouse or growth chamber of comparable size is prohibitive. The breeder must either limit the genetic material that is grown or use an alternative off-season environment.

Growing conditions in a greenhouse or growth chamber can cause atypical growth, even though field conditions for plant growth are duplicated as closely as possible. For example, soybean plants generally grow excessively tall in the greenhouse, which prevents adequate assessment of most agronomic characters. Certain genotypes of soybean will not consistently produce seed when planted in the greenhouse during some winter months.

Location at Low Latitudes. Locations where plants can be grown in the field throughout the year are commonly used as off-season environments. They often are referred to as winter nurseries, although some breeders use a location throughout the year. The locations commonly used by breeders in temperate climates of the United States include Florida, Arizona, Hawaii, Puerto Rico, and Mexico.

Off-season environments in low latitudes are popular because more space is available than in a greenhouse or growth chamber. They are used extensively for hybridization to form breeding populations. In crops such as maize, testcross seed may be produced to evaluate individuals for combining ability. Off-season environments in low latitudes are widely used for inbreeding of both self- and cross-pollinated species. Selection may be practiced for certain characters. Seed increases are made of genotypes for further evaluation or for production of commercial quantities of a cultivar.

The disadvantages of a location at a low latitude depend on its location and the quality of personnel available to conduct the work. It is relatively easy to underestimate the amount of work required to conduct a quality off-season nursery. It often is more difficult to grow a crop in an off-season environment at a low latitude than in a more temperate climate.

Any nursery located some distance from a breeder's station has certain inherent disadvantages. The breeder must move to the location to supervise the breeding operations or must hire qualified persons to do so. Travel to the location can be time-consuming and expensive.

Locations at low latitudes may be subject to production hazards not normally encountered in a more temperate climate. Undesirably low temperatures may occur sporadically at some locations. Crops have been destroyed by frost in Florida. Soybean hybridization is not possible during the winter when temperatures are consistently below 16°C. This has prevented hybridization during certain winter months in Florida and Puerto Rico. Pests commonly are found at a low latitude that are not of importance in a temperate climate. It may be necessary to spray a crop regularly with fungicides and insecticides to obtain satisfactory plant growth and seed quality. High temperatures and humidity during seed maturation in tropical environments can drastically reduce seed quality. The germination of soybean seed declines rapidly if seed is not harvested immediately after it is mature. This necessitates more timely harvest than is necessary in the cooler climates of high latitudes. Additional hazards that have been encountered include salt injury, bird damage, and hurricanes.

The movement of seed to locations in lower latitudes may be subject to quarantine regulations. The regulations are established to prevent the movement of pests from one area to another. They may require that seed or other plant parts be treated in a special manner before shipment, which may be time-consuming and expensive. The plant material must be inspected by authorized individuals, a process that can delay its shipment. Soybeans grown in Puerto Rico were subject to quarantine regulations after soybean rust, a disease not found in the United States, was discovered on the island. The regulation during the early 1980s required that seed shipped from the location be free of any debris, treated with a special fungicide, and inspected.

Locations in an Opposite Hemisphere. Some crops are grown commercially during different months in the northern and southern hemispheres. This makes it possible to grow two generations each year under conditions that are favorable

for many breeding operations. In the development of cultivars for the northern United States, breeding material is grown in the northern hemisphere May through October and in the southern hemisphere November through April. Conversely, breeding programs in the southern hemisphere can use the northern hemisphere as an off-season environment.

Breeding material grown in an off-season environment in another hemisphere is subject to the same conditions as the commercial crop. Land area suitable for growing the breeding material generally is not a limiting factor. Hybridization and inbreeding can be readily accomplished and evaluation of important characteristics frequently is possible. Large-scale seed increase that involves commercial equipment can be accomplished in both hemispheres.

Despite the advantages of growing breeding material in another hemisphere, this is not done as commonly as using locations in lower latitudes, for several reasons. It generally is more time-consuming and expensive for travel and shipment of plant material to another hemisphere. The time interval from harvest in one hemisphere to planting in another is often short, which can complicate preparation of material for planting. Only one crop can be grown in the off-season. At locations of low latitude, plants have a shorter generation length, which often makes it possible to obtain two generations during the off-season. Soybeans adapted to the northern United States have a 90-day generation length when grown at low latitudes; therefore, two generations can be grown during the off-season from November through May. When grown in South America, only one generation of soybean can be obtained from November through May.

Production of breeding material in another hemisphere has some of the same restraints as the use of a location at a low latitude. Proper supervision of the breeding material may be difficult. The pests common to one hemisphere may not be the same as those in another. The movement of seed may be subject to quarantine regulations.

Selection Intensity (k)

The chance of obtaining a superior segregate increases as the number of lines tested is augmented. The selection intensity associated with population improvement can increase as the number of lines tested increases.

The relationship between selection intensity and number of lines tested can be illustrated by assuming that 20 superior lines from those evaluated will be used as parents for recombination. If 100 lines are tested, the selection intensity will be 20 percent and the value of $k = 1.40$ (Table 17-5). With doubling of the number of lines tested, the selection intensity decreases to 10 percent and k increases to 1.75. Evaluation of 400 lines would result in a selection intensity of 5 percent and a k of 2.06.

Another way to illustrate the importance of number of lines tested is to assume that the selection intensity will be a constant value of 10 percent. If 100 lines are tested, the top 10 percent of the lines (10) can be used as parents. With

200 lines, the top 10 percent represents 20 parents. Use of a greater number of lines as parents may reduce the amount of inbreeding in the population (Chap. 8).

Plant breeders are constantly seeking ways to increase the number of genotypes evaluated without sacrificing the quality of the testing program. The use of computers and the mechanization of field research have increased markedly the number of genotypes that can be effectively evaluated compared with use of hand labor. These aspects of cultivar development are discussed in Chap. 19.

Parental Control (c)

Parental control can be increased from $^1/_2$ to 1 by selecting a character before female plants have been pollinated by both selected and unselected males (Table 17-1). Control of both female and male parents is preferred because the alleles passed to the next generation are from selected individuals. If only the female is controlled, only one-half of the alleles (those of the egg cells) are selected. The other half of the alleles are from unselected pollen that does not contribute to genetic gain.

The parental control can be doubled from 1 to 2 by using selfed seed or clones to recombine individuals with superior half-sib progeny, instead of using remnant half-sib seed. The alleles present in selfed seed are from only the selected individuals. The alleles in half-sib seed include alleles from the selected individual and alleles from the population when the seed was developed. Parental control of all alleles (selfed seed) is superior to parental control of only one-half of the alleles (remnant half-sib seed).

Genetic Variability (σ_A^2, σ_g^2)

The amount of additive genetic variance in a population is influenced by the (a) genetic diversity of the parents, (b) amount of inbreeding before individuals or families are evaluated, (c) type of individual or family evaluated, and (d) number of generations of recombination between cycles of selection.

Genetic Diversity of the Parents. Genetic diversity is influenced by the number of parents used to develop a population and their ancestry. In a diploid species, a single-cross population can possess only two alternative alleles at a locus, one from each parent. Each additional parent used to develop a population (three-way, double-cross) has the potential of contributing additional alleles and, therefore, additional genetic variability. This principle is especially important when developing a population in which to conduct recurrent selection for multiple cycles. The potential progress to be realized by selection is limited by the number of alleles in the base population (cycle 0). The greater the number of parents

used for recombination each cycle of selection, the greater the potential genetic variability available for selection.

Genetic diversity is a function of the ancestry of the parents. The alleles contributed by two parents with different ancestry are more likely to vary than those contributed by parents with a common background. Breeders consider the ancestry of parents when developing populations. In a recurrent selection program, the decrease in genetic variability that occurs through inbreeding can be reduced by selecting lines as parents each cycle that trace to different crosses or S_0 plants.

There is considerable debate about the value of using exotic parents for increasing the genetic diversity of a population. "Exotic" refers to any germplasm that is not highly productive in the area for which new cultivars are being developed. There is a possibility that exotic parents can contribute alleles for improvement that are not available in adapted germplasm. It also is highly likely that they will contribute many undesirable alleles to the population. As a result, an increase in genetic variability of a population developed with exotic parents generally is associated with a reduction in the population mean compared with the mean of a population developed from adapted parents. An increase in genetic variability that is due to the presence of more inferior segregates is of no value to the breeder for the selection of improved cultivars. For that reason, most breeders do not use exotic parents for populations from which they expect to obtain new cultivars in a short time. Exotic parents are sometimes used for populations that are intended for improvement by a long-term recurrent selection program.

Amount of Inbreeding Before Evaluation. Additive genetic variability is associated with the frequency of homozygous loci in a population of individuals. The effect of inbreeding (F) on the amount of σ_A^2 is described by the equations in Table 17-4. For example, the amount of σ_A^2 among selfed lines is determined by the equation $(1 + F)\sigma_A^2$. An F_2 population ($F = 0$) has σ_A^2 and a population of doubled haploids with no heterozygous loci ($F = 1$) has $2\sigma_A^2$. The effect of inbreeding on genetic gain is illustrated in Table 17-9. The gain per cycle for $S_{0,1}$ lines ($F = 0$) was 10.8 and for $S_{3,4}$ lines ($F = 0.875$) was 16.2.

The value of increasing σ_A^2 by inbreeding must include consideration of the time required to obtain more genotypes with a greater level of homozygosity. The importance of this principle is illustrated in Table 17-9 by the comparison of genetic gain utilizing $S_{0,1}$ or $S_{3,4}$ lines. Although genetic gain per cycle was greater with $S_{3,4}$ lines, the additional time (y) required per cycle caused the genetic gain per year to be greater for $S_{0,1}$ lines, regardless of the number of seasons per year. This principle is an important consideration in selecting an appropriate breeding strategy for cultivar improvement.

Number of Generations of Recombination Between Cycles. The amount of genetic variability in a population is associated with the number of opportunities

for recombination among members of the population. Recombination between a pair of linked genes requires that both loci be heterozygous. A crossover in the genotype $\frac{AB}{ab}$ will produce *Ab* and *aB* gametes, but a crossover in the genotypes $\frac{AB}{AB}$ or $\frac{ab}{ab}$ cannot produce the *Ab* or *aB* combinations. The probability of recombination between linked genes increases with each generation of random mating in a population. This principle is considered in determining the number of generations of intercrossing to be conducted in developing a population.

The number of generations of intercrossing can influence the genetic gain per year by increasing the number of seasons required per cycle. The types of seasons available to the breeder will be an important consideration in selecting the number of generations of intercrossing. By conducting intercrossing in a season not suited for character evaluation, the breeder may be able to add a generation of intercrossing without influencing genetic gain per year.

Within-Plot Variability (σ^2_u, σ^2_{wg}, σ^2_w)

The variability within plots (σ^2_w) is determined by environmental effects (σ^2_u) and genetic segregation (σ^2_{wg}). Their impact is a function of the number of plants that are averaged together to determine a plot mean. This can be expressed as: $\frac{\sigma^2_w}{n} = (\sigma^2_u + \sigma^2_{wg})/n$, where n is the number of plants per plot.

The value of n is 1 for individual plant selection in a population, such as for recurrent phenotypic selection. In line or family evaluation, the value of n is a function of the size of plot and the plant population used, expressed as plants per plot. The effect of increasing plants per plot can be estimated by holding σ^2_w constant and varying the value of n. For example, assume that σ^2_w equals 700.

n	$\sqrt{\sigma^2_w/n}$
1	26.5
2	18.7
3	15.3
4	13.2
5	11.8
10	8.4
20	5.9
30	4.8
40	4.2
50	3.7
60	3.4
100	2.6

The value of adding an additional plant per plot decreased as n increases. The difference between 1 and 10 plants per plot in our example was $26.5 - 8.4 = 18.1$ units. The difference between 60 and 100 plants per plot was only 0.8 units. The breeder can estimate the most efficient number of plants per plot for each of the characters to be evaluated.

Plot-to-Plot Variation (σ^2)

The estimate of σ^2 is associated with environmental differences from one plot to another. Its magnitude is influenced by the uniformity of plots within a replication. In field experiments, σ^2 is likely to increase as the amount of land area in a replication increases, because of soil heterogeneity. Possibilities for reduction of σ^2 are decreasing the number of plots per replication and decreasing the size of plots per entry.

For selection of single plants, plot-to-plot variation is not a factor if the plants within a plot or grid instead of plants in different plots are compared. For example, modified ear-to-row selection involves single plant selection within superior half-sib families. The equation for predicting genetic gain for within-plot selection does not include the plot-to-plot component (σ) in the denominator because selection is within a plot, not among plots (Table 17-2). For recurrent phenotypic selection, the plot-to-plot variance does not occur in the denominator when a population is subdivided into blocks in a grid (Table 17-2).

Experimental Error (σ_e^2)

A reduction in σ_u^2, σ_{wg}^2, and σ^2 causes a decrease in σ_e^2, because $\sigma_e^2 = (\sigma_u^2 + \sigma_{wg}^2)/n + \sigma^2$. The impact of σ_e^2 also is influenced by the number of replications (r) and environments (t) of testing, as reflected by the expression σ_e^2/rt. The relative importance of number of replications versus number of environments will be discussed in the next section.

Genotype × Environment Interaction (σ_{ge}^2)

The impact of the genotype × environment interaction can be reduced by evaluating the lines in multiple environments (t), expressed as σ_{ge}^2/t. The breeder must choose the relationship between number of replications and environments that will give the most genetic gain with the least cost.

The effect of different numbers of replications and environments can be estimated from the expression $(\sigma_e^2/rt) + (\sigma_{ge}^2/t)$. Increasing the number of environments has a greater effect than increasing replications, because t is a divisor for both σ_e^2 and σ_{ge}^2.

If the number of plots that could be grown was fixed, cost was not a factor,

and σ^2_{gr} was important, the greatest genetic gain would be realized by growing one replication at many environments. In practice, this generally is not possible, because the cost of using different environments is more than the cost of growing additional replications at an environment. Each additional replication or environment that is used will decrease the phenotypic variance, but the amount will decrease as r and t increase. The principle is the same as increasing the number of plants to reduce σ^2_w.

Indirect Selection

The efficiency of cultivar development would be improved by the identification before hybridization of parents that would produce superior progeny, of characters that would permit efficient indirect selection for yield and other desirable economic traits, and of characters that influence performance in particular environments. Consider a breeding program whose objective is to develop a cultivar with improved yield potential for an environment with high temperature and low moisture. The breeder would like to know if there are characters other than yield per se that can be used to select parents and identify the segregates that will have the desired performance. These might include leaf size and orientation, plant height, branching or tillering, length of the seed-filling period, root depth, photosynthetic rate, leaf temperature, and transpiration rate.

A quantitative character such as yield is the culmination of plant processes that begin with germination of a seed or the initiation of a vegetative propagule. The physiological processes may have a direct or indirect influence on the final yield that is obtained. Selection for variation among genotypes for physiological characters may enhance selection for yield per se.

The character of ultimate importance in a selection program can be referred to as the primary character (Falconer, 1981). The characters that influence the primary character are referred to as secondary characters. For example, yield may be considered a primary character and photosynthetic rate, length of the seed-filling period, and root depth as secondary characters.

The potential value of indirect selection for a secondary character that is quantitatively inherited was summarized by Falconer (1981) in the equation

$$\frac{CR_x}{R_x} = r_A \frac{i_y h_y}{i_x h_x}$$

where CR_x = amount of improvement in primary character obtained by indirect selection for secondary character

R_x = amount of improvement obtained by direct selection for primary character

r_A = genetic correlation between primary character (x) and secondary character (y)

i_y = selection intensity for secondary character

i_x = selection intensity for primary character

h_y = square root of narrow-sense heritability of secondary character

h_x = square root of narrow-sense heritability of primary character

The equation defines the factors that must be considered for effective indirect selection of morphological and physiological traits for improvement of characters of economic importance.

Genetic Correlation Between Characters. Selection for morphological or physiological characters is of no value if the characters' performance is not correlated with performance of the primary character. Determination of the genetic correlation between characters requires adequate evaluation of appropriate genetic material over a number of environments. Some studies on trait associations have utilized isolines, i.e., lines that are genetically similar except for genes controlling a character of interest (Qualset et al., 1965). It may be possible to create variability within a cultivar for a trait through physical rather than genetic manipulations. Pendleton and colleagues (1968) mechanically manipulated leaf angle in maize plants to investigate the relationship of this character with yield. The disadvantage of using physical manipulation is that the behavior of altered plants may not be representative of normal plant behavior.

Because isolines are often not available and physical manipulation is often not possible or desirable, initial experiments on trait associations commonly involve the evaluation of selected cultivars or experimental lines that differ for the traits of interest. A sufficient number of lines differing in the traits may be available or may be derived through hybridization and selection. The phenotypic correlations obtained from such experiments provide a preliminary indication of the association between characters. Definitive evaluation of the genetic correlation requires the use of random genotypes from segregating populations to obtain the necessary variance and covariance estimates.

Selection Intensity. Selection intensity is a ratio of the number of genotypes selected divided by the number of genotypes tested. The number of genotypes that can be evaluated for a secondary character compared with a primary character has an important influence on the effectiveness of indirect selection. Secondary characters that are more expensive and difficult to measure than the primary character are less likely to be useful than those that increase the number of genotypes that can be evaluated.

Heritability. The effectiveness of indirect selection is enhanced when the secondary character has a higher heritability than that of the primary character. The higher heritability may be associated with greater additive genetic variability, less environmental variation, less genotype × environment interaction, or lower nonadditive genetic variability. The effectiveness of indirect selection is based

Table 17-11 Plant Characters with Potential Value in Ideotype Breeding for Barley, Oat, Rice, and Wheat

Leaf Characters	Culm Characters	Inflorescence Characters
Leaf size	Number of culms	Awn length
Leaf angle	Survival of culms	Awn number
Number of leaves	Diameter of culms	Kernel number
Duration of leaves	Number of ears	Kernel weight
Thickness of leaves	Vascular bundles	
Specific leaf weight		
Stomatal frequency		
Type of Canopy	**Root Characters**	**Other**
Height of plants	Volume	Photoperiod response
Harvest index	Depth	Length of growth stages
Angle of ear		

Source: Rasmusson and Gengenbach, 1983.

on the square root of the heritabilities. As a result, the heritability of the secondary character must be considerably larger than that of the primary character to increase the ratio substantially. A heritability of 0.9 for the secondary character and 0.45 for the primary character represents a ratio of 2, but the ratio of the square root of the heritabilities is only 1.4.

Ideotype Breeding

Some plant breeders believe that a model plant type can be specified for a crop species in terms of morphological and physiological characters. This model plant type is commonly referred to as an ideotype. Rasmusson and Gengenbach (1983) have presented a list of plant characters with potential value in ideotype breeding (Table 17-11). The ideotype developed for a crop species should be subject to change as new information on plant physiology becomes available or as new methods of crop production are adopted.

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CHAPTER EIGHTEEN

Genotype × Environment Interaction

Cultivars of a crop are grown under a wide range of conditions. They are exposed to different soil types, soil fertility levels, moisture levels, temperatures, and cultural practices. All of the variables encountered in producing a crop can be described collectively as the environment.

When cultivars are compared in different environments, their performance relative to each other may not be the same. One cultivar may have the highest yield in some environments and a second cultivar may excel in others. Changes in the relative performance of genotypes across different environments are referred to as genotype × environment interaction.

TYPES OF INTERACTIONS

Every factor that is a part of the environment of a plant has the potential to cause differential performance that is associated with genotype × environment interaction. Environmental variables can be classified as either predictable or unpredictable factors (Allard and Bradshaw, 1964). Predictable factors are those that occur in a systematic manner or are under human control, such as soil type, planting date, row spacing, plant population, and rates of nutrient application. Unpredictable factors are those that fluctuate inconsistently, including rainfall, temperature, and relative humidity.

Predictable factors can be evaluated individually and collectively for their interaction with genotypes. Studies have been made of genotype × soil type, genotype × row spacing, genotype × planting date, and genotype × plant population interactions.

Unpredictable factors contribute to the interactions of genotypes with loca-

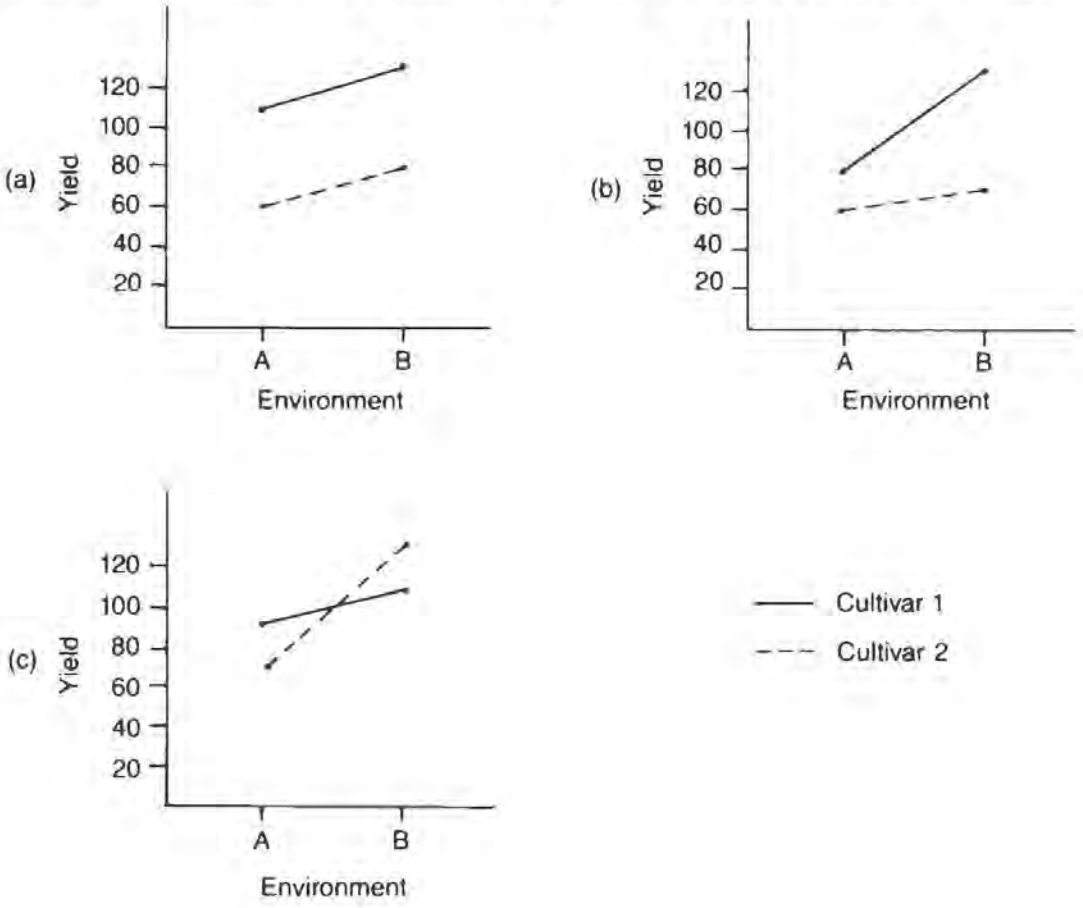
tions and years. Genotype × location, genotype × year, and genotype × location × year interactions have been evaluated in many crop species.

The relative performance of genotypes across environments determines the importance of an interaction. There is no genotype × environment interaction when the relative performance among genotypes remains constant across environments. In Fig. 18-1a, cultivar 1 has the same yield superiority over cultivar 2 across two environments. No genotype × environment interaction is present because the yield differential between the cultivars is 50 units in both environments.

Genotype × environment interactions can occur in two ways.

1. The difference among genotypes can vary without any alteration in their rank. In Fig. 18-1b, a genotype × environment interaction is present because cultivar 1 yields 20 units more than cultivar 2 in environment A and 50 units more in environment B.

Figure 18-1 The relative performance of two cultivars in two environments. (a) No genotype × environment interaction is present. (b) Genotype × environment interaction is present but does not alter genotypic ranking. (c) Genotype × environment interaction is present and alters genotypic ranking.



2. The rank among cultivars may change across environments. In Fig. 18-1c, cultivar 1 is more productive in environment A, but cultivar 2 is more productive in environment B. The change in rank between cultivars results in a genotype × environment interaction. The most important genotype × environment interaction for the plant breeder is one caused by changes in rank among genotypes.

Genotype × environment interactions are of interest to breeders for several reasons.

1. The need to develop cultivars for specific purposes is determined by an understanding of the interaction of genotypes with predictable environmental factors. Unique cultivars may be required for different row spacings, soil types, or planting dates.
2. The potential need for unique cultivars in different geographical areas requires an understanding of genotype × location interactions. The importance of this interaction can determine if division of a large geographical area into subareas is needed for testing new genotypes and obtaining data on cultivar performance for crop producers.
3. Effective allocation of resources for testing genotypes across locations and years is based on the relative importance of genotype × location, genotype × year, and genotype × location × year interactions.
4. The response of genotypes to variable productivity levels among environments provides an understanding of their stability of performance. An understanding of the environmental stability of genotypes helps in determination of their suitability for the fluctuations in growing conditions that are likely to be encountered.

ASSESSMENT OF GENOTYPE × ENVIRONMENT INTERACTIONS

Determining the importance of genotype × environment interactions requires appropriate experimental procedures. An understanding of the steps involved in the design, conduct, analysis, and interpretation of such an experiment can be useful.

Experimental Design

Objective. Planning of any experiment begins with a statement of the concept or hypothesis to be evaluated, sometimes phrased in the form of a question. Is the relative performance among genotypes different when they are grown with use of conservation tillage versus conventional tillage? Do genotypes respond differently to high and low rates of inorganic nitrogen fertilization? The breeder may have a hypothesis about the answer to the question on the basis of practical

experience. It is critical that the hypothesis should not be regarded as fact, an attitude that can bias the interpretation of the experimental results.

Genotypes for Evaluation. The genotypes chosen for an assessment of possible interactions are an important consideration in design of the experiment. Some analyses of genotype × environment interaction are not based on an experiment specifically designed for that purpose, particularly the assessment of the importance of interactions with locations and years. Instead, breeders utilize data from cultivars and experimental lines that have been evaluated over locations and years as a part of normal testing programs. The main disadvantage of such an approach is that the cultivars and experimental lines may not be a random sample of available genotypes. Estimates of genotype × environment interaction obtained with selected genotypes may be higher or lower than those that would be obtained with random individuals. The preferred procedure is to use a random sample of genotypes from those that are available for testing.

Tests must be conducted at two or more locations and years to obtain estimates of genotype × location, genotype × year, and genotype × location × year interactions (Table 18-1). The locations of testing generally are those routinely used by the breeder. Locations may be considered a fixed effect when they are not randomly chosen from all possible sites in an area. Some breeders consider them a random effect, however, because the breeder has no control over the climatic conditions that will occur at a location in any year. For the same reason, years of testing are considered random effects.

At least two replications are needed in each location and year to obtain an estimate of experimental error with which to test the significance of the interactions of interest. Any additional replications will provide a more reliable estimate of the experimental error.

An example of an experiment designed to assess genotype × environment interaction was a study of tobacco in North Carolina by Jones and colleagues (1960) (Table 18-2). They used seven cultivars that had been included in the official state trials for tobacco at five locations during each of 3 years. The seven cultivars differed for agronomic characteristics, disease resistance, and chemical composition. The five locations were those used routinely for tobacco evaluation. They had been selected to represent the tobacco production area of North Carolina and differed in soil type, elevation, and climatic conditions. The cultivars, years, and locations studied were considered representative samples of each variable and were designated as random effects.

Data Analysis

Data analysis includes the calculation of mean values, determination of the statistical significance of the sources of variation, and calculation of estimates of appropriate variance components (Snedecor and Cochran, 1980; Steel and Torrie, 1980).

Table 18-1 Analyses of Variance for Experiments in an Annual Crop with Different Numbers of Locations and Years

Sources of Variation	Degrees of Freedom	Expected Mean Squares
One location in one year:		
Replications	$r-1$	—
Genotypes	$g-1$	$\sigma_e^2 + r(\sigma_g^2 + \sigma_{gl}^2 + \sigma_{gy}^2 + \sigma_{gly}^2)$
Error	$(r-1)(g-1)$	σ_e^2
One location in two or more years:		
Years	$y-1$	—
Replications in years	$y(r-1)$	—
Genotypes	$g-1$	$\sigma_e^2 + r(\sigma_{gy}^2 + \sigma_{gly}^2) + ry(\sigma_g^2 + \sigma_{gl}^2)$
Genotypes × years	$(g-1)(y-1)$	$\sigma_e^2 + r(\sigma_{gy}^2 + \sigma_{gly}^2)$
Error	$y(r-1)(g-1)$	σ_e^2
One year at two or more locations:		
Locations	$l-1$	—
Replications in locations	$l(r-1)$	—
Genotypes	$g-1$	$\sigma_e^2 + r(\sigma_{gl}^2 + \sigma_{gly}^2) + rl(\sigma_g^2 + \sigma_{gy}^2)$
Genotypes × locations	$(g-1)(l-1)$	$\sigma_e^2 + r(\sigma_{gl}^2 + \sigma_{gly}^2)$
Error	$l(r-1)(g-1)$	σ_e^2
Two or more locations in two or more years:		
Years	$y-1$	—
Locations	$l-1$	—
Replications in years and locations	$yl(r-1)$	—
Years × locations	$(y-1)(l-1)$	—
Genotypes	$g-1$	$\sigma_e^2 + r\sigma_{gly}^2 + ry\sigma_{gl}^2 + rl\sigma_{gy}^2 + rly\sigma_g^2$
Genotypes × years	$(g-1)(y-1)$	$\sigma_e^2 + r\sigma_{gly}^2 + rl\sigma_{gy}^2$
Genotypes × locations	$(g-1)(l-1)$	$\sigma_e^2 + r\sigma_{gly}^2 + ry\sigma_{gl}^2$
Genotypes × years × locations	$(g-1)(y-1)(l-1)$	$\sigma_e^2 + r\sigma_{gly}^2$
Error	$yl(r-1)(g-1)$	σ_e^2

Source: Johnson et al., 1955.

The sources of variation in an experiment are partitioned into main effects and their interactions (Table 18-1). The mean squares for the sources of variation are determined, and appropriate F-tests are made to assess the probability that a source of variation is significant. Components of variance can be calculated for the main effect of genotype and its interactions with years and locations. Standard errors can be computed for each component of variance.

Data Interpretation

Data interpretation includes consideration of the statistical significance of sources of variation and an assessment of the practical importance of variation observed among mean values. The genotype × location interaction measures the consistency of performance among genotypes at different locations. The consistency of performance of genotypes in different years is indicated by the genotype × year interaction. The genotype × location × year interaction measures the consistency of performance among genotypes for each combination of year and location. An experiment conducted at two locations in 2 years has four year–location combinations: year 1–location 1, year 1–location 2, year 2–location 1, and year 2–location 2. A significant genotype × location × year interaction indicates that the relative performance among genotypes was not the same for each of the year–location combinations. For all of the just mentioned interactions, an examination of mean values is necessary to determine if a significant interaction is due to a change in rank among genotypes or to changes in the differences among genotypes without variation in rank (Fig. 18-1).

The lack of any statistically significant interactions involving genotypes simplifies the nature of the testing program required for cultivar development and simplifies cultivar selection by the producer. Theoretically, the lack of a significant interaction of genotypes with locations, years, or location × year indicates that a test at one location during one year would be sufficient to identify genotypes with superior genetic potential. Cultivars with the best performance at one location in one year would also be superior at other locations in other years.

The practical implications of statistically significant genotype × environment interactions depend on the cause of the interaction. Genotype × environment interactions are not a problem for the breeder or producer if they are not due to changes in rank of performance among genotypes. Under these circumstances, a test at one location in 1 year could be used to identify superior genotypes, if genetic differences among lines were adequately expressed. The same cultivars would be superior in all locations and years, although the amount of superiority would vary. Significant genotype × environment interactions that involve changes in rank are common. In determining the practical implication of the interactions, the breeder must consider the extent of the changes in rank and their potential impact on genetic improvement. Subjective judgments often must be made; therefore, two breeders evaluating the same data may adopt different courses of action. The options available to the breeder are different for each type of interaction.

Genotype × Location. Wide fluctuations in the rank performance of genotypes at test locations suggest that it may be desirable to develop genotypes for different locations through independent selection and testing programs. The cost of establishing independent programs for different geographical areas is substantial; therefore, the decision can be difficult. Before establishing independent breeding

programs, the breeder should make a detailed examination of the environmental factors responsible for the genotype × location interaction. If the differences among locations are due to soil type or other factors that are consistent from year to year, independent programs may be appropriate. Temporary differences among locations associated with unusual climatic conditions would not justify independent programs.

Another consideration in determining the implications of genotype × location interaction is that fluctuations in rank may not preclude selection of superior genotypes for multiple locations. Assume that a group of genotypes was divided into three classes: good, intermediate, and poor. A genotype × location interaction could be caused by fluctuations in rank among genotypes within the three groups, but not among groups. Such an interaction would be unlikely to justify the establishment of breeding programs for independent locations, at least for the initial stages of testing.

Genotype × Year. An inconsistent ranking among genotypes grown in different years is in some regards more difficult to deal with than a genotype × location interaction. A breeder does not have the option of establishing independent breeding programs for different years. The primary option available is to identify genotypes that exhibit superior performance on the average across years. This involves the testing of genotypes in several years before selection of one for release as a cultivar. To reduce the length of time for genetic improvement, multiple locations in 1 year often are used as a substitute for years. The substitution is only effective when the divergence in climatic conditions among locations is comparable to differences among years.

Genotype × Year × Location. When there are fluctuations in the ranking of genotypes associated with individual location–year combinations, the breeder must identify genotypes with superior average performance over locations and years. This can be accomplished by testing over multiple locations and years. For example, an analysis of genotype × environment interaction for tobacco yield in North Carolina indicated that the mean squares for the genotype × year and genotype × location interactions were not significant (Jones et al., 1960). The rankings among cultivars were similar each year when averaged over locations (Table 18-2). Rankings of cultivars were also similar at each location when averaged over years. But the genotype × year × location interaction was significant in the experiment. The interaction seemed to be associated with specific conditions, such as rainfall pattern and disease infestation, that caused the ranking of cultivars to vary among certain year–location combinations. If the cultivar with the highest average performance over years is chosen, it would be expected to have acceptable performance the next year, but it may not be the best in that particular season. Producers often reduce the effect of fluctuations caused by genotype × year interaction by growing more than one cultivar each season.

Table 18-2 Yield per Acre and Relative Yield Ranking of Seven Tobacco Cultivars Averaged Over Five Locations for 3 Years*

	1955		1956		1957	
Cultivar	Pounds	Rank	Pounds	Rank	Pounds	Rank
C 139	2231	1	2306	1	2179	2
DB 244	1978	2	2069	2	2218	1
C 140	1830	3	1980	3	1865	3
Hicks	1701	4	1901	5	1735	5
402	1635	5	1777	7	1665	7
DB 101	1623	6	1819	6	1695	6
Va. 21	1622	7	1941	4	1809	4

*The cultivar × year interaction was not significant.

Source: Jones et al., 1960.

SELECTION OF LOCATIONS FOR TESTING

The selection of locations for the evaluation of a quantitative character is an important decision for the plant breeder, and involves a number of considerations. Locations generally are chosen that are representative of the area where a new cultivar will be grown commercially. The cost of transporting machinery and personnel may influence the distance of a location from the main research center. The availability of suitable land may be a factor when the size of the test area is large.

A primary consideration in site selection is the diversity of environments that can be obtained within a year. This is particularly important when cultivars are desired that perform well in a range of environments. A breeder will attempt to use test locations that have environments as diverse as those that would be encountered at one location in 2 or more years.

Several statistical procedures have been developed to characterize the similarity of environments encountered at different locations. They are based on the similarity in the relative performance of a group of genotypes that have been evaluated in replicated tests at all locations of interest.

Analysis of Variance

The similarity in relative performance of genotypes can be determined by the magnitude of the genotype × location interaction computed by a standard analysis of variance (Horner and Frey, 1957). The locations used for testing can be grouped into combinations of two or more. The genotype × location interactions computed for the various combinations of locations can be compared to determine the similarity or diversity of the locations involved.

The analysis of variance procedure was used by Horner and Frey (1957) to evaluate the possibility of dividing the state of Iowa into subareas for oat cultivar recommendations. Cultivar × location interactions were determined for various combinations of nine locations from which yield data were available during a 5-year period. The combinations with the lowest cultivar × location mean squares were considered the most suitable as subareas within Iowa. Horner and Frey suggested that the state could be divided into four subareas for testing.

Correlation Among Locations

Guitard (1960) used a diallel design for correlations between locations to determine the relative performance of barley cultivars over locations. The performance of the cultivars grown at one location was correlated with their performance at each of the other locations. Guitard found that by grouping locations with similar cultivar responses, he could reduce the number of locations used for yield tests from ten to five with only a small loss of information.

Cluster Analysis

Cluster analysis has been used to classify locations into groups within which genotype × location interactions are not significant. Locations are successively grouped on the basis of similarity in their interaction with a set of genotypes. At each level of clustering, an analysis of variance can be performed to test for significance of interactions. Ghaderi and colleagues (1980) used cluster analysis to investigate the interaction of genotypes of wheat at eight locations in Michigan. Although the genotype × location interaction was found to be significant over all locations, it was not significant within a cluster of the seven most similar locations. On the basis of results of cluster analysis, Barker and co-workers (1981) suggested that the performance of reed canarygrass clones grown in Iowa was representative of their performance in Minnesota and Wisconsin.

ALLOCATION OF RESOURCES

An understanding of genotype × environment interactions is useful for determining the optimum allocation of resources for testing.

An assessment of resource allocation requires data from a group of genotypes grown at two or more locations during 2 or more years. The analysis of variance provides estimates of the variance components associated with error (σ_e^2), genotype × location × year (σ_{gly}^2), genotype × location ((σ_{gl}^2), genotype × year

(σ^2_{gy}), and genotypes (σ^2_g). These can be used to compare different allocations of resources.

Variance of a Genotype Mean

The ability to identify significant differences among genotypes increases as the variance of the genotype mean decreases. Jones and colleagues (1960) used the concept of variance of a genotype mean to compare different strategies for plot allocation in tobacco trials (Table 18-2). The symbols they used have been modified in the following equation to conform to those used in this book.

$$V_{\bar{x}} = \frac{\sigma^2_e}{rly} + \frac{\sigma^2_{gly}}{ly} + \frac{\sigma^2_{gl}}{l} + \frac{\sigma^2_{gy}}{y}$$

The values for replications (r), locations (l), and years (y) were varied. The calculated variances of a genotype mean ($V_{\bar{x}}$) were compared with that obtained with their previous allocation of plots that included 2 years, five locations, and four replications. They concluded that 2 years, five locations, and three replications would be a more acceptable allocation of resources for their testing program.

Genetic Gain

Resource allocation for yield trials of maize was evaluated by Sprague and Federer (1951) by the calculation of genetic gain. The formula for genetic gain that they presented was similar in principle to the equation used in Chap. 17.

$$G_s = \frac{k\,\sigma^2_g}{\sqrt{(\sigma^2_e/rly) + (\sigma^2_{gly}/ly) + (\sigma^2_{gl}/l) + (\sigma^2_{gy}/y) + \sigma^2_g}}$$

Genetic improvement with various resource allocation procedures can be expressed in terms of gain per year (G_y) by dividing the genetic gain per cycle by the number of years required to complete a cycle of selection, $G_y = G_s/y$. Genetic gain per year is useful for evaluating resource allocation because it takes into account the length of time involved in evaluating genotypes for release as new cultivars.

Heritability

The effect of resource allocation on genetic gain can be assessed by its alteration of heritability. Heritability (h^2) can be expressed as

$$h^2 = \frac{\sigma^2_g}{(\sigma^2_e/rly) + (\sigma^2_{gly}/ly) + (\sigma^2_{gl}/l) + (\sigma^2_{gy}/y) + \sigma^2_g}$$

Rasmusson and Glass (1967) used this equation to derive heritabilities from estimates of variance components and various numbers of replications, years, and locations. The heritabilities of seven traits in two barley populations were found to vary considerably among the hypothetical testing methods.

Cost Associated with Resource Allocation

The cost associated with replications and locations is an important consideration in the allocation of resources. A fixed number of plots often is available for evaluating a genotype. In the absence of significant genotype × environment interactions, increasing the number of replications at a single location is as effective in improving gain as increasing the number of years or locations. If σ^2_{gl} and σ^2_{gly} are greater than zero, the amount of genetic improvement will be greatest with a maximum number of locations and minimum number of replications at each location. The cost of the genetic improvement generally will be increased, however, when the number of locations is increased. A compromise between the cost and the amount of genetic improvement may have to be reached.

The cost of genetic improvement was examined by Sprague and Federer (1951) for yield tests of maize. They calculated the cost per plot as a function of the number of plots per location and the cost of transportation. They indicated that cost per unit of genetic gain was least when one location was used, because transportation costs were eliminated. They also demonstrated, however, that the cost per plot decreased rapidly as the number of plots per location increased. Their cost for 25 plots at a location was less than half the cost for 100 plots at a location. The lower cost was achieved by dividing the expense for transportation among more plots. By using a sufficiently large number of plots per location, they were able to reduce the difference in cost per unit of genetic gain with varying numbers of locations.

Cost assessments may vary considerably among crops and breeding programs. The cost analysis by Sprague and Federer for maize did not apply to the situation in tobacco described by Jones and colleagues (1960) (Table 18-2). Data collection for tobacco in North Carolina was not influenced by the cost of transportation because personnel living on existing research stations provided most of the labor. As a result, the cost of a plot was essentially the same regardless of the location in which it was utilized.

Time Considerations in Resource Allocation

Genotype × year and genotype × location × year interactions often are significant for yield and other quantitative characters. Each additional year of evaluation will increase the reliability of information concerning the performance of a genotype. In terms of the statistical procedures discussed, each additional year

will reduce the theoretical variance of a genotype mean, increase the total genetic gain, and increase heritability.

There are practical limits, however, to the number of years of testing that can be conducted before a decision must be made about the genetic value of an individual. For recurrent selection programs, an increase in the number of years of testing may increase genetic gain per cycle but decrease genetic gain per year. A decision on the release of a genotype as a cultivar cannot be postponed indefinitely.

Most breeding programs attempt to save time by substituting additional locations for years of testing. The substitution is not on a one-for-one basis when the genotype × location component is less than that of genotype × year. Public breeding programs for many crops have a cooperative arrangement for testing that permits a large number of locations to be used each year at minimal cost. Private companies accomplish the same objective by establishing research stations in different geographical areas. Each station conducts tests of genotypes at several locations in a designated region.

STABILITY OF GENOTYPE PERFORMANCE

The reliability of cultivar performance across locations and years can be an important consideration in plant breeding. Some cultivars are adapted to a broad range of environmental conditions, while others are more limited in their potential distribution. There are cultivars that perform similarly regardless of the productivity level of the environment, and others whose performance is directly related to the productivity potential of the environment.

The stability of cultivar performance across environments is influenced by the genotype of individual plants and the genetic relationship among plants of the cultivar. The terms homeostasis and buffering have been used to describe the stability in performance of individual plants or groups of plants over different environments.

The terms developmental homeostasis and individual buffering have been used to describe the stability of individual plants (Allard and Bradshaw, 1964; Briggs and Knowles, 1967). It has been shown that heterozygous individuals, such as F_1 hybrids, are more stable than their homozygous parents. The stability of heterozygous individuals seems to be related to their ability to perform better under stress conditions than homozygous plants.

The terms genetic homeostasis and population buffering have been used to describe the stability of a group of plants that exceeds that of its individual members. (Allard and Bradshaw, 1964; Lerner, 1954). Heterogeneous cultivars generally have more stability on the average than do homogeneous ones.

Methods of Stability Analysis

A number of statistical procedures have been developed to enhance our understanding of genotype × environment interaction and its relationship to stability.

Analysis of Variance. The environmental stability of a group of genotypes has been evaluated with standard analysis of variance procedures. The significance of interactions involving genotypes is determined with an F-test. The relative magnitude of the genotype × location, genotype × year, and genotype × location × year variance components can be used to determine the effect of locations and years on the stability of a group of genotypes.

The relative environmental stability of different groups of genotypes has been compared with use of the analysis of variance procedure. Sprague and Federer (1951) found genotype × location and genotype × year interactions to be of greater significance in maize single-cross hybrids than in double-cross hybrids.

Pairwise Analysis of Variance. The standard analysis of variance procedure for a group of genotypes does not provide information on the environmental stability of individual genotypes. Information on individual genotypes can be obtained by conducting a combined analysis of variance for every pairwise combination of genotypes at all locations in a given year (Plaisted and Peterson, 1959). For each genotype, the mean of σ^2_{gl} estimates derived from its combination with all other genotypes can be calculated. These means provide a measure of the contribution of each genotype to the genotype × location interaction.

Regression Analysis. The environmental stability of individual genotypes has been estimated by the use of regression analysis (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966). A group of genotypes is grown over a range of environments. The mean performance of the genotypes at each environment is referred to as the environmental index. The performance of each genotype is regressed on the environmental index to obtain its mean performance over all environments, its linear response to varying environments, and an estimate of deviations from linear regression at the individual environments. A desirable genotype was described by Eberhart and Russell (1966) as one with a high mean, a regression coefficient of 1.0, and deviations from regression of 0. Such a genotype would have increased performance as the productivity of the environment improves.

Geometric Analysis. Hanson (1970) has proposed a measure of genotypic stability based on deviations from expected yield over environments. These deviations define the coordinates of a genotype within a stability space having a number of dimensions equal to the number of environments. Genotypic stability is expressed as a euclidean distance, either from a stable genotype (relative stability) or between any two genotypes (comparative stability).

Cluster Analysis. Cluster analysis also has been used to classify genotypic stability. On the basis of similarities in phenotypic responses in 16 environments, Ghaderi and colleagues (1980) arbitrarily grouped winter wheat genotypes into 10 clusters. They concluded that this method was effective in identifying groups of genotypes with various combinations of means and stabilities.

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CHAPTER NINETEEN

Field-Plot Techniques

The fundamental purpose of plant breeding is to identify genotypes with superior performance in commercial production. A large proportion of the time and expense devoted to cultivar development is in field evaluation of breeding material. The tests may involve genotypes in an initial stage of evaluation or those being given final consideration for release as new cultivars. The characters evaluated range from those that can be measured readily by visual examination to those that must be measured with appropriate instruments. The genetic potential of a genotype for some characters may be determined effectively with one or a few plants in a small plot, while for other characters extensive evaluation in larger plots may be needed.

It is the responsibility of the plant breeder to select the field-plot techniques that will provide the maximum amount of information with the resources available. The challenge is to adequately test as many genotypes as possible. The resources available to plant breeders vary; usually several alternative techniques are available for character evaluation. Plant breeders must decide which techniques will be the most effective and efficient in their particular situation.

Detailed discussions of field-plot techniques and data analysis are provided by Gomez and Gomez (1984) and LeClerg et al. (1962). An overview of the general principles will be provided in this chapter.

SOURCES OF VARIATION

The ideal way to compare genotypes would be to grow all of them in exactly the same environment and to measure their characteristics in precisely the same manner. The differences among genotypes in this ideal situation would be due only to variation in their genetic potential; therefore, the best genotype could be chosen without error. This ideal is impossible to achieve under field conditions because of lack of uniformity in the environment to which the genotypes are

exposed. Nevertheless, the use of appropriate field-plot techniques can maximize the accuracy with which genotypes are compared and selected.

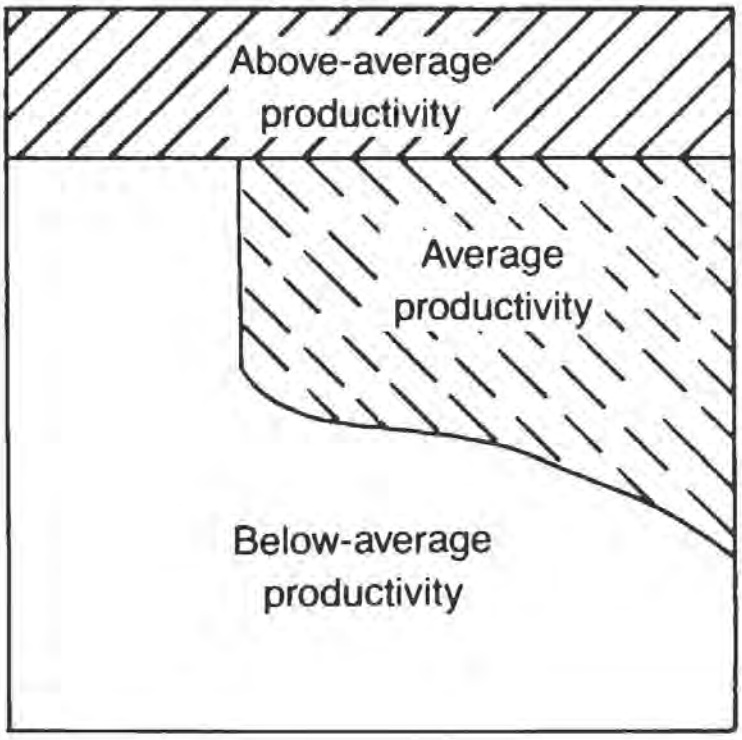
The factors that result in test conditions that are less than ideal can be referred to collectively as sources of experimental error. They include variation in the environment to which each genotype is exposed and lack of uniformity in the measurement of characters. The breeder has opportunities to minimize experimental error by carefully selecting the site to be used for field trials, the cultural practices used in crop production, the plot size and shape, and the method of data collection.

Site Selection

Variation in the productivity of the soil is commonly referred to as soil heterogeneity (Fig. 19-1). Causes of soil heterogeneity include variation in soil type, availability of plant nutrients, and soil moisture. The variation cannot be completely eliminated, but it often can be minimized by careful selection of the area in a field where plots will be grown. Soil maps are helpful for understanding the variation in soil type that is present. Soil types differ in their inherent ability to retain nutrients and moisture. Entire trials or at least an entire replication should be grown on a single soil type whenever possible.

Visual inspection of a field is important, even when a soil map is available.

Figure 19-1 Example of potential variation in soil productivity in a test area.



When a field has been identified a year in advance as a potential test site, it is useful for the breeder to look for variability in productivity of the crop grown in the area. The breeder should note variation in the terrain that may cause water to accumulate more in one place than in another. Differences in soil tillage after harvest of the previous crop may be observed that could result in nonuniformity of the area. Uneven distribution of plant or animal waste on a field should be noted as a potential contributor to variation in the availability of plant nutrients.

Before a site is chosen, information should be obtained on cultural practices that were followed in the production of previous crops, with special attention to the application of chemicals that could influence the crop that the breeder will be evaluating. The residue from herbicides applied for control of weeds in previous crops may cause damage to the crop to be tested. The following quotation from a research article by Thorne and Fehr (1970b) on soybean breeding illustrates the importance of herbicide residue:

> The strains were evaluated at Ames and Kanawha, Iowa, in 1968. . . . At Kanawha, part of the experiment was inadvertently planted in a field treated with atrazine herbicide two years before. All plots in the area were destroyed.

Previous cultural practices in a field can be especially important at research stations where crops are rotated from one field to another on a systematic basis. The research conducted on crops previously grown on a field can influence markedly the uniformity of the test site. For example, plots of oats were planted in a field at the Agronomy Research Center of Iowa State University in which soybeans had been planted the previous year. Growth of the oats varied in strips, as if nitrogen fertilizer had been applied unevenly to the field. A review of the previous soybean research revealed that the strips of oats with extra growth coincided with areas where mature soybeans had been cut and left unthreshed. The nitrogen in the soybean seeds in the strips was available to the oats the following year, and caused nonuniformity of nutrient availability in the test site.

Cultural Practices

Experimental error can be minimized by the use of uniform cultural practices for production of the crop being tested. Chemicals should be applied uniformly to the test site before, during, or after planting. Uneven soil compaction should be minimized during tillage operations. Application of supplemental water by irrigation may reduce variability in soil moisture. Weed control should be uniform; most breeders try to eliminate all weeds during the growing season to avoid experimental error caused by differential weed competition.

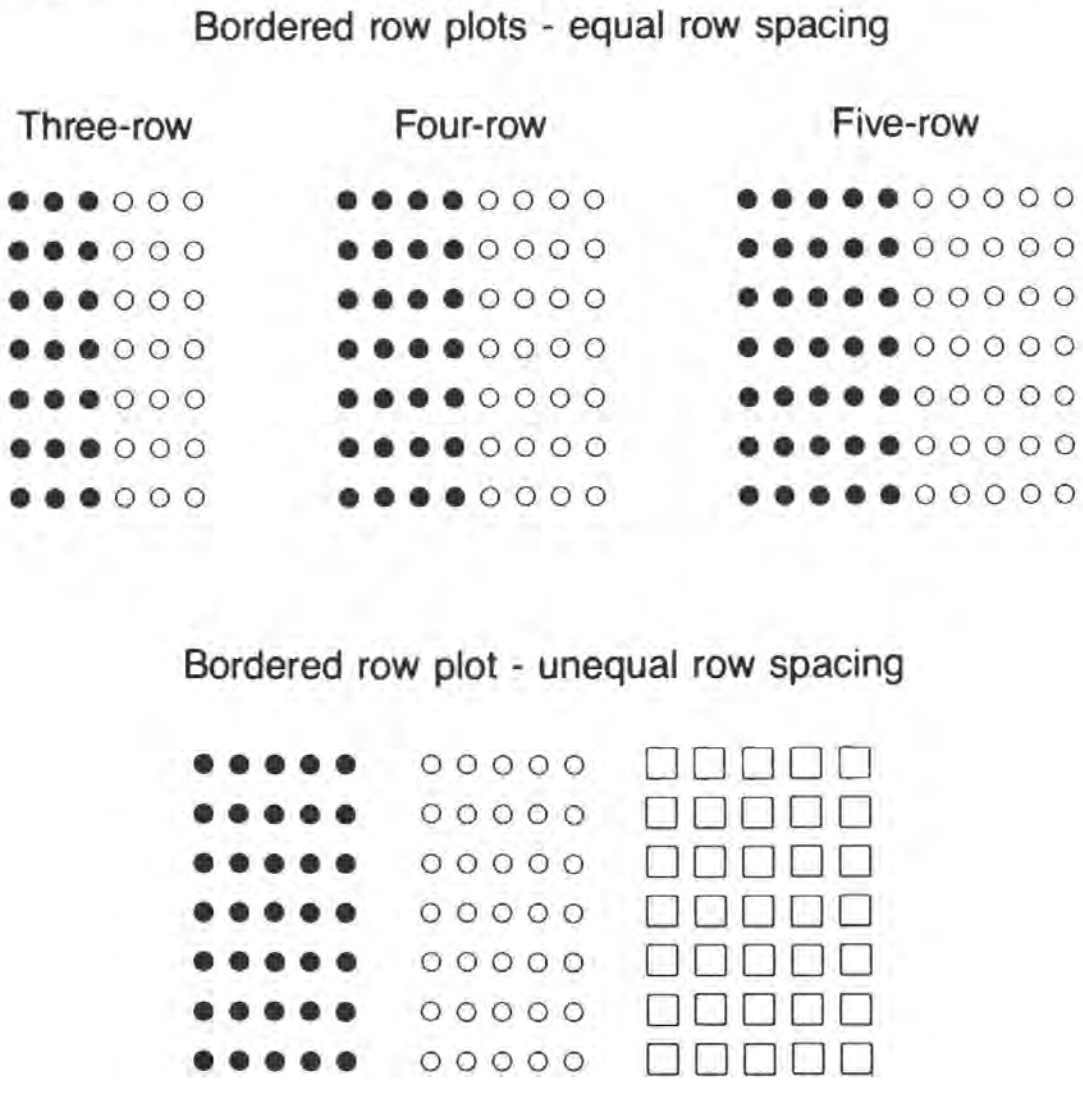
The development of equipment specifically designed for planting, managing, and harvesting research plots has permitted breeders to grow plots more efficiently. The emphasis in the design and use of any equipment must be on the uniformity with which genotypes are handled.

Plot Type

Experimental error increases whenever interplot competition causes the performance of a genotype in one plot to be altered by the performance of genotypes in adjacent plots. Interplot competition results primarily from intergenotypic competition, which is the differential ability of genotypes to compete with each other. Interplot competition is more important for the evaluation of some characters than for others. It is only through appropriate experimentation that a plot type can be identified that will provide reliable information for the character of interest.

The effects of interplot competition can be avoided by the use of plots with multiple rows in which only plants in the center rows are evaluated (Fig. 19-2). In plots with three or more rows, the outermost rows are designated as the border or guard rows. The function of the border rows is to prevent plants in adjacent

Figure 19-2 Illustration of bordered row plots with different cultivars designated as ●, ○, and □. (Courtesy of Fehr, 1978.)

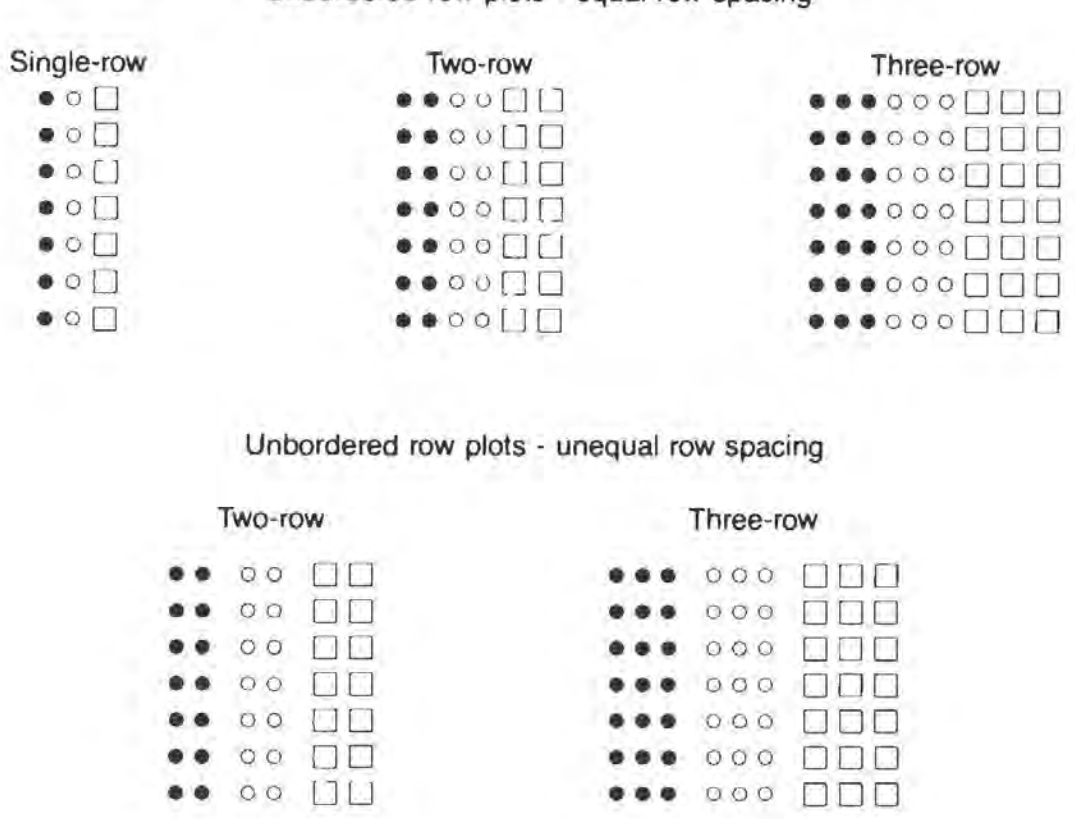


plots from influencing the performance of plants in the center of the plot. Each bordered plot can be considered a miniature field that is unaffected by neighboring fields. The spacing between plots can be greater than the within-plot spacing to facilitate the movement of equipment, particularly when narrow rows are utilized.

It would be ideal if bordered plots could be used for the evaluation of all characters that are influenced by interplot competition. That ideal is difficult to achieve when thousands of genotypes are being evaluated. Bordered plots require seed and land that do not directly provide data for a genotype. Borders take up two-thirds of the seed and land area for three-row plots and one-half for four-row plots. The cost and availability of seed and land often necessitate restriction of the use of bordered plots to the evaluation of genotypes that are being given final consideration for release as cultivars.

Interplot competition can be reduced, but not eliminated, with unbordered plots of two or more rows, all of which are used to evaluate a character (Fig. 19-3). A genotype in a single-row plot is subjected to interplot competition on both sides. Interplot competition is reduced by one-half in plots with two rows, two-thirds with three rows, three-fourths with four rows, and four-fifths with five rows. The estimated reduction of interplot competition with increasing numbers of rows is based on the fact that each row of a plot must compete on two sides. The border rows are each subjected to interplot competition on one side

Figure 19-3 Illustration of unbordered row plots with different cultivars designated as ●, ○, and □. (Courtesy of Fehr, 1978.)



but not on the other. Any rows within the two border rows are protected from interplot competition. This can be expressed as

$$\text{Reduction in interplot competition compared with single-row plot} = \frac{(\text{number of rows per plot} \times 2 \text{ sides}) - 2 \text{ sides}}{\text{number of rows per plot} \times 2 \text{ sides}}$$

$$\text{Two-row plot} = \frac{(2 \times 2) - 2}{2 \times 2} = 1/2$$

$$\text{Three-row plot} = \frac{(3 \times 2) - 2}{3 \times 2} = 2/3$$

The amount of interplot competition also can be reduced by increasing the spacing between rows of adjacent plots. Interplot competition in soybeans was evaluated with five cultivars grown in single rows spaced 100, 75, 50, and 25 cm apart (Gedge et al., 1977). The average effect of interplot competition on seed yield was 2.6 percent for the 100-cm spacing, 5.3 percent for 75 cm, 8.0 percent for 50 cm, and 17.6 percent for 25 cm.

A combination of increased row spacing between plots and a large number of rows can minimize interplot competition in unbordered plots. In the soybean example of the preceding paragraph, the average change in yield for single-row plots spaced 100 cm apart was 2.6 percent. The percentage theoretically would be reduced to 1.3 percent for two-row plots and to 0.9 percent for three-row plots. Rows within a plot are not subjected to interplot competition; therefore, the spacing between rows within a plot can be less than the spacing between adjacent plots. Figure 19-3 illustrates a two-row plot in which the spacing between plots is wide enough to minimize interplot competition and the spacing within the plot is reduced to minimize the land area required for each plot.

Some breeders plant one cultivar as a common border between one- or two-row plots. In barley, a lodging-resistant cultivar is used as a common border to prevent genotypes with lodging susceptibility from falling on genotypes in adjacent plots, thereby causing them to lodge unnaturally. The use of a common border has been evaluated as a means of eliminating intergenotypic competition between plots for seed yield and other quantitative characters. The results of the research indicate that a common border can reduce but not eliminate interplot competition (Thorne and Fehr, 1970a). The average interplot competition for seed yield among four soybean cultivars in single-row plots spaced 50 cm apart was compared with competition of the cultivars when a common border was used (Gedge et al., 1977). Interplot competition averaged 11.0 percent in single-row plots and 8.3 percent in plots with a common border.

Plot Size and Shape

The size of plots used to evaluate genotypes varies with the character being evaluated, the amount of experimental error that is considered acceptable for

measuring a character, the experimental design, and the growth characteristics of the crop. Plots vary in size from those for a single plant that is harvested by hand to those that are wide and long enough to be harvested with the same equipment used by farmers for commercial production.

Single-Plant Plots. Individual plants commonly are evaluated in segregating populations. There is no replication of the individuals, unless vegetative propagation of clones is possible. The spacing among plots varies with the crop species involved. Gardner (1961) spaced individuals 50 by 100 cm apart when selecting for yield in maize. Burton (1974) spaced plants of a population of Pensacola bahiagrass 60 by 60 cm apart when conducting recurrent phenotypic selection for forage yield. Burton and Brim (1981) used a 46 by 46 cm spacing among soybean plants for selection of oil composition in the seed.

Single-plant plots are used for the replicated evaluation of experimental lines or cultivars by the honeycomb field design (Fasoulas, 1979). The number of plants evaluated for a line is equal to the number of replications in the experiment. Fasoulas (1981) indicated that 100 single-plant plots (replications) per line would provide satisfactory results. The plots of the lines in a test are organized in a systematic manner to permit comparison of a plant of one line with adjacent plants of other lines (Fig. 19-4). The honeycomb design has not been adopted by plant breeders for replicated evaluation of lines because it requires more labor and is less amenable to mechanization than microplots or conventional row plots.

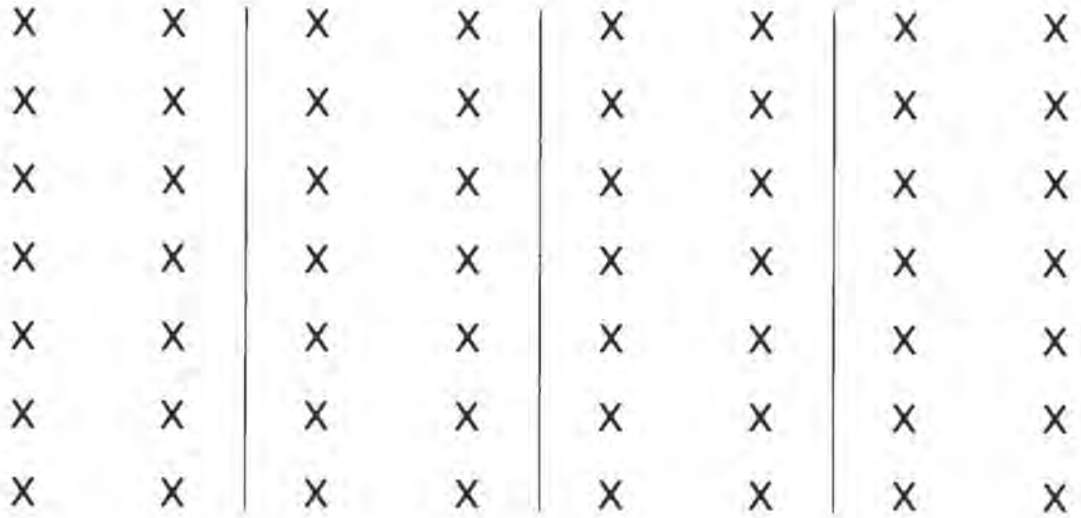
Multiple-Plant Plots. The evaluation of experimental lines or cultivars by plant breeders is usually done in plots containing two or more plants. Plot size varies from small microplots consisting of a hill or short row to a plot with one or more rows several meters in length.

Microplots. Microplots are used to minimize the amount of seed or land required to evaluate a group of lines. In an unbordered microplot, the effects of interplot competition must be considered when determining an appropriate distance among plots (Fig. 19-5). For oats, hill plots spaced about 30 by 30 cm apart have been used (Frey, 1965), while for soybeans, a spacing of about 1 by 1 m is more common (Garland and Fehr, 1981).

The number of plants in a microplot differs among crops. A planting rate of 30 seeds per hill is satisfactory in oats (Frey, 1965), while a rate of 12 seeds per hill is used for soybeans (Garland and Fehr, 1981). When short rows are used as microplots, the plant density is comparable to that of larger row plots.

There is a lack of agreement among plant breeders concerning the effectiveness of microplots for evaluation of agronomic characters, particularly seed yield. Breeders who use microplots indicate that they are useful for eliminating inferior lines during the first year of yield evaluation. Lines with acceptable performance in microplots are evaluated in conventional row plots during subsequent years of testing, to identify those that merit release as cultivars (Frey,

Grid design



Honeycomb

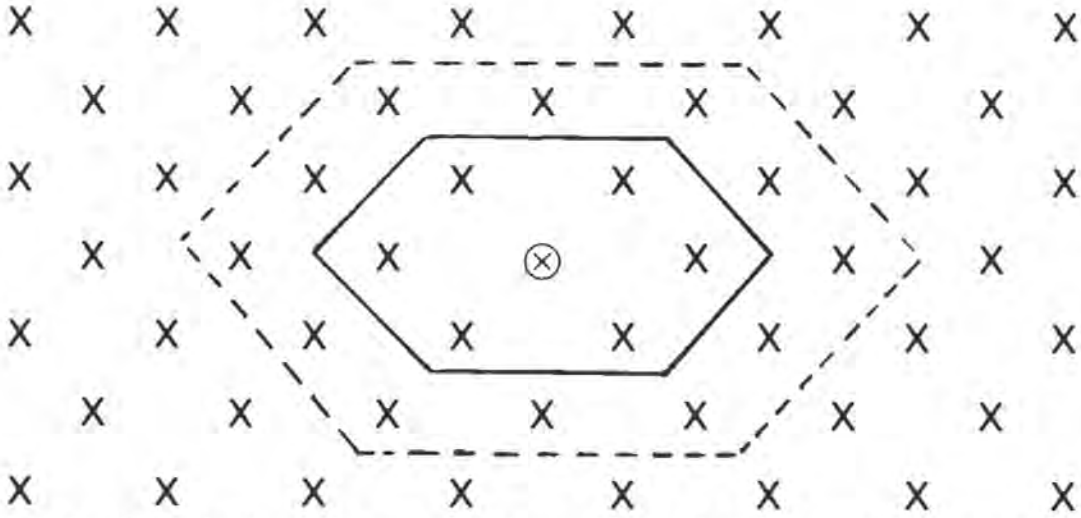


Figure 19-4 Grid and honeycomb design to select individual plants in a population. For the grid design, plants are divided into blocks and the best ones chosen from each (Gardner, 1961). For the honeycomb design, the plant at the center of the hexagon, ⊗, is compared with every other plant within the hexagon (Fasoulas, 1979). A plant is chosen only if it is superior to every other plant in the hexagon. The hexagons outlined represent two different selection intensities.

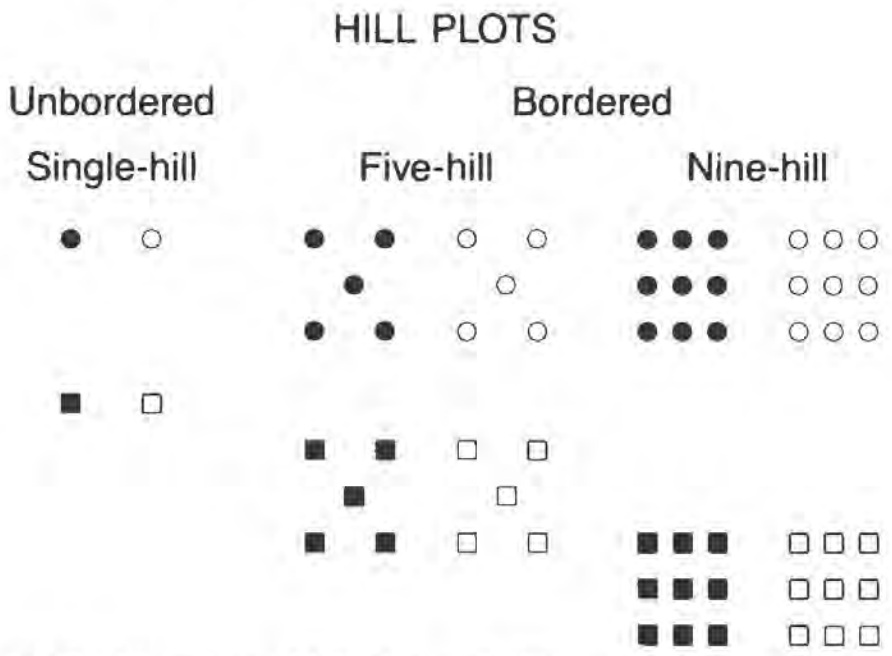


Figure 19-5 Illustration of hill plots with different cultivars designated as □, ○, ●, and ■ (Fehr, 1978).

1965; Garland and Fehr, 1981). The advantages of microplots compared with conventional row plots for the first year of yield testing are that less land is required per plot and that enough seed for replicated tests can be obtained from a single plant, which eliminates a season for seed increase. Breeders who do not use microplots are concerned about the reliability of yield data obtained from them. The coefficients of variability for microplots generally are about one and one-half to two times larger than for conventional row plots.

Row Plots. Row plots are used by virtually all plant breeders for replicated testing of genotypes. The overall plot size is determined by the number of rows, the spacing between rows, and the row length.

Single-row plots of 1 to 2 m in length are widely used for the visual evaluation of characters. Many breeders evaluate lines on the basis of their appearance in small unreplicated plots, and advance the desirable ones to replicated tests the following season. Visual selection and seed increase commonly are accomplished with the same plot.

A plot used to evaluate the yield of lines for the first time often is smaller than that employed for advanced stages of evaluation. For advanced yield tests, the breeder attempts to use a plot size that approaches or equals the dimensions considered optimal for the crop species involved. Optimum plot size is the minimum land area required to measure a character with an acceptable level of experimental error.

Optimum plot size can be determined by the use of data from a uniformity trial (Cochran, 1937). A single cultivar is planted as a solid stand, without alleys,

in an area representative of that used for yield evaluation. The cultural practices used to produce the crop are the same as those used for yield trials. The area is subdivided into small units, and the seeds or plants from each unit are harvested and weighed separately. Experimental error associated with plots of different size can be determined by making various combinations of the small units.

Optimum plot size also is determined through practical experience. The breeder often will experiment with plots of different size to find the smallest one that has an acceptable level of experimental error. Breeders often do not agree on what they consider acceptable experimental error; consequently, an optimum size for one person may not be optimum for another.

Plot width generally is determined by considerations other than the relationship of shape to experimental error. The primary factors are the number of rows required to minimize or avoid interplot competition and the width of the planting and harvesting equipment that is available. Plot width influences the percentage of land area that must be devoted to alleys between plots. Long, narrow plots require a lower percentage of alley space than do wide, short plots. This advantage is offset in bordered plots because the percentage of land area devoted to border rows decreases as the number of rows per plot increases.

Plot length provides flexibility for plot size. Before calculators and computers became readily available, row length in the United States was varied to obtain a plot size that was a fraction of an acre (one-tenth, one-twentieth, etc.) to simplify the conversion of plot yields to yields per acre. With use of computers for data summarization and analysis, this is no longer necessary.

Data Collection

The experimental error associated with the evaluation of a character is influenced by measurement errors during data collection. For characters evaluated visually, experimental error occurs whenever the data collector fails to give an identical rating to plots with an identical appearance. Reliability of the evaluation can be established readily by rating a series of plots at different times and comparing the ratings. It is essentially impossible to give visual ratings without error; therefore, the breeder must decide when the error is acceptable and when it is so large that genetic differences will be masked.

Some characters can only be evaluated efficiently with the use of an appropriate machine or instrument. Experimental error can occur because of failure to prepare a plot properly for measurement, of not obtaining a representative sample of the plot for evaluation, of using nonuniform procedures for sample preparation, and of failure of the machine or instrument to operate properly.

Preparation of a plot for data collection may begin before planting. For experimental error to be reduced, the seeds or plants of every genotype used for planting must be treated equally. If seeds or plants of genotypes to be compared

do not come from a common environment, environmental error may result. Lint yield and seedling vigor of a cotton cultivar were found to differ in plots grown from seeds obtained from different locations (Peacock and Hawkins, 1970). Seed source also has been shown to influence seed yield of soybeans (Fehr and Probst, 1971.)

In some crop species, uniformity of plant density among plots can be important in minimizing experimental error. With maize, it is a common practice to thin yield test plots to a uniform stand soon after seedling emergence. Thinning is not considered necessary with some crop species, particularly those that have the ability to branch or tiller in response to low plant density, such as barley and wheat. It also is a common practice with crops such as maize to record the number of plants per plot immediately before harvest. The yield of the plots is adjusted for plant density by an analysis of covariance, to minimize experimental error in the comparison of genotypes.

When a blank alley is used at the end of row plots, the end plants generally are more productive than those growing in the center of the plot. When end plants are harvested, yield of the plot is inflated in comparison to the yield obtained from plants growing in the center of the plot. This inflation will prevent a direct comparison of plot yields with those expected in a normal commercial planting, unless an appropriate adjustment is made for all plots. The adjustment may be made by considering the alley as part of the plot area; therefore, plot length is the distance from the center of one alley to the center of the next, instead of the distance between plants at opposite ends of a row. For example, if the length of row containing plants is 5 m and the alley is 1 m wide, the plot length for computing plot area is considered to be 6 m.

The yield inflation by end plants in a plot does not contribute to experimental error unless genotypes in a test do not respond similarly to the space in the alley. The experimental error associated with differential response of genotypes to an alley can be minimized by adjusting yields according to characteristics of the genotypes that influence this response. The end plants of soybean genotypes with late maturity give a greater yield inflation than do genotypes of early maturity. Values have been developed with which to adjust plot yields for maturity of soybean genotypes (Wilcox, 1970). More commonly, comparisons among soybean genotypes are restricted to those of similar maturity, unless plots are end-trimmed before harvest.

The only way to eliminate yield inflation by end plants is to remove the plants before harvest. This procedure, referred to as end-trimming, is a standard procedure with some crops. The end plants are removed late enough in plant development that the remaining plants in the plot cannot take advantage of the extra space. The length of row removed from each end of the plot must be long enough to include all plants that have benefited from the space provided by the alley. In soybean, 0.6 m is removed from each end of the plot (Wilcox, 1970).

The problem of a blank alley is minimized in some crops by planting the

alley with rows of a single genotype perpendicular to the test plots. The result is that the plants at the end of a plot must compete with plants in the alley, and thus their yield may not be inflated as much as is the case with a blank alley. Plants in the alley are removed immediately before the plots are harvested.

EXPERIMENTAL DESIGNS

The arrangement of genotypes in a field experiment is referred to as the experimental design. Some of the designs utilized to compare genotypes are common to research in many disciplines. Others have been developed to deal with the problem of comparing a large number of genotypes as inexpensively as possible. The experimental designs used for the initial evaluation of a large number of genotypes often differ from those used in the advanced stages of testing a few select genotypes. Alternative designs will be considered here for comparison of single plants, unreplicated genotypes in multiple-plant plots, and replicated genotypes.

Single-Plant Selection

The first evaluation step in the development of a cultivar generally is the selection of individual plants from a population. Individual plant selection also is employed in population improvement by recurrent phenotypic selection.

When single-plant selection in a population is for characters with a high heritability, the plants generally are grown in a random order and those with desirable characteristics are selected. Cultivars may be grown in adjacent plots to serve as standards with which to evaluate single plants. Date of flowering, plant height, time of maturity, and certain types of pest resistance are examples of characters for which single plants are selected without any predetermined arrangement of the individuals. They represent characteristics that are not strongly influenced by environmental variation.

Single-plant selection in a population grown in a relatively large land area can be hampered seriously by soil heterogeneity for characters with a low heritability, such as seed or plant yield. Figure 19-1 illustrates variation in soil productivity in an area where a population of plants may be grown. If plants with the highest yield are selected regardless of their location in the field, those in the area of above-average productivity will be favored. A plant with outstanding genetic potential that is located in the area with below-average productivity may be discarded. Two experimental designs are available that minimize the effect of soil heterogeneity by comparing plants that are most adjacent to each other.

Grid Design. Gardner (1961) proposed that the land area on which a population of individual plants is grown can be subdivided into blocks or grids of a limited

area (Fig. 19-4). Plants within each block are compared with each other, and the superior ones are selected. Comparisons are not made between plants from different blocks. This experimental design has been well accepted by plant breeders, particularly those conducting recurrent phenotypic selection for yield or other characters with a low heritability.

Honeycomb Design. Fasoulas (1973) developed a honeycomb design for selecting individual plants in a population (Fig. 19-4). Five aspects of the design and its implementation are unique. (a) Seeds or clones are spaced equidistantly from each other in a hexagon pattern. The name of the design was chosen because the hexagon patterns resemble a honeycomb of bees. (b) Plants are spaced far enough apart that they do not compete with adjacent individuals. At the appropriate spacing for a species, a missing plant does not influence the performance of adjacent individuals, because each plant already has sufficient space in which to develop to its full potential. (c) Homogeneous check cultivars can be included for comparison, if desired. Every plant of the check is compared with a different group of plants in the population. (d) The size of the hexagon used to select single plants determines the selection intensity in the population. The effect of soil heterogeneity is minimized because only those plants within the area of the hexagon are compared. (e) Every plant in the population is evaluated by placing it in the center of the hexagon. A plant is chosen only if it is superior to every other plant in the hexagon. By moving the hexagon, every plant is compared with a different group of plants in the population.

Comparison of the Grid and Honeycomb Designs. Both the grid and honeycomb designs reduce the problem of soil heterogeneity in the selection of characters of low heritability. In a comparison of the designs, the advantages of one are the disadvantages of the other, and vice versa.

There are three primary advantages of the grid design.

1. The spacing of plants does not have to be in a precise pattern. This facilitates the use of conventional plot equipment for planting and cultivation. Mechanized planting of the honeycomb design would require specialized equipment.
2. Selection intensity can be varied by altering the number of plants in a block and the number of plants selected. Only certain selection intensities are possible with the honeycomb design.
3. Use of a defined area for each block facilitates visual comparison of plants for selection. It is possible to compare plants within a block visually and collect data only from those with the best potential. Use of the moving hexagon for the honeycomb design makes it impractical to compare each plant with appropriate ones in its hexagon; therefore, data must be recorded for every plant, except those that are obviously inferior.

The honeycomb design has two advantages compared with the grid design.

1. Homogeneous check cultivars can be included to permit comparisons of individual plants with a standard. When one-seventh of the plants are a check, they can be arranged so that every plant in the population can be compared with a check plant. To provide adjacent plants of one check cultivar in a grid system, one-third of the area would have to be devoted to the check.
2. More than two check cultivars can be included readily in hexagons of 19 or more plants. Use of two or more check cultivars in the grid system would require that a large fraction of each block be devoted to check plants.

Unreplicated Evaluation with Multiple-Plant Plots

Plant breeders routinely conduct visual selection among lines in unreplicated plots for maturity, disease resistance, standability, and other characters of high heritability. Evaluation for yield in a single replication has been used to a limited extent to eliminate inferior lines before initiation of expensive replicated tests. With a single replication, each line is compared once with check cultivars or other lines to determine its genetic potential. A number of different arrangements are available for estimating the genetic potential of lines. One method is to compare each line with a common check cultivar (Baker and McKenzie, 1967). Figure 19-6 represents a hypothetical example of the yield of six lines in a single replication. In the figure, the yield of each line is expressed as a percentage of the yield of the check cultivar immediately adjacent to it.

Another alternative is to express the yield of each line as a percentage of the weighted average of the adjacent check plot and of the check plot two plots removed. The purpose for using a weighted average is to minimize the potential problem caused by an unusually poor yield of a check plot. In Fig. 19-6, the check cultivar adjacent to lines B and C has a much lower yield than other check cultivars. This results in an extremely high percentage for lines A and B. The weighted average of check cultivars could be computed as

$$(\tfrac{2}{3} \times \text{yield of adjacent check}) + (\tfrac{1}{3} \times \text{yield of check two plots removed}) = \text{weighted average of check cultivars}$$

The percentage yield of each line is computed as

$$\text{Line A} = \frac{59}{(\frac{2}{3} \times 55) + (\frac{1}{3} \times 39)} \times 100 = 119$$

$$\text{Line B} = \frac{70}{(\frac{2}{3} \times 39) + (\frac{1}{3} \times 55)} \times 100 = 158$$

$$\text{Line C} = \frac{53}{(\frac{2}{3} \times 39) + (\frac{1}{3} \times 48)} \times 100 = 126$$

$$\text{Line D} = \frac{51}{(\frac{2}{3} \times 48) + (\frac{1}{3} \times 39)} \times 100 = 113$$

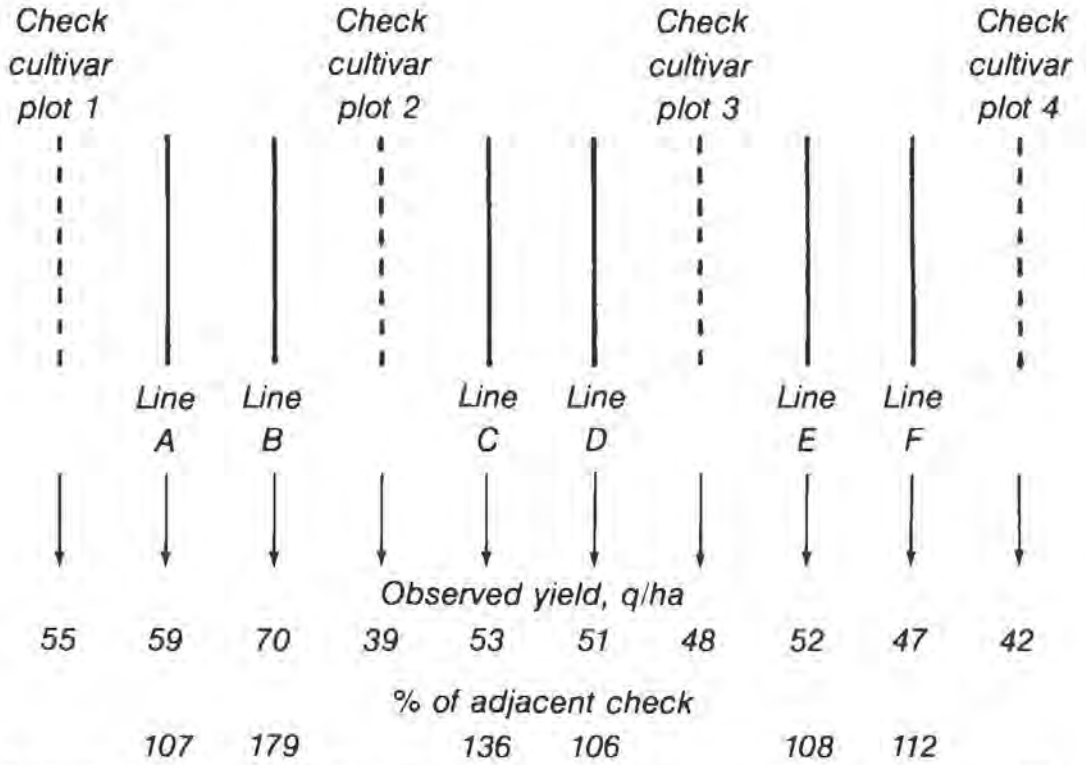


Figure 19-6 One possible arrangement of lines in a single-replication test. The performance of each line is computed as a percentage of the performance of the common check cultivar adjacent to it. Line B would be considered the superior one.

$$\text{Line E} = \frac{52}{(\frac{2}{3} \times 48) + (\frac{1}{3} \times 42)} \times 100 = 113$$

$$\text{Line F} = \frac{47}{(\frac{2}{3} \times 42) + (\frac{1}{3} \times 48)} \times 100 = 107$$

Another method used to compare genotypes in single replications is the moving mean (Mak et al., 1978; Townley-Smith and Hurd, 1973). Each genotype is compared with adjacent test genotypes, not with a check cultivar.

The disadvantage of single-replication tests is that the breeder has only one plot value with which to assess the genetic potential of a line. If by chance a line is placed on a plot of soil with above-average productivity, relative to that of plots with which the line is compared, it will seem to be genetically superior, even though it may not be. In replicated tests, the breeder will have more than one plot with which to evaluate each line. For this reason, single replications are not commonly used for yield evaluation.

Replicated Tests

Two or more independent comparisons of lines in a test provide a means of estimating whether variation in performance among lines is due to differences in genetic potential or to environmental variation. Each comparison is as rep-

lication. Replication can be accomplished by growing two or more plots of each line at one or more locations or one plot at each of two or more locations or years.

Randomization. One important consideration in the arrangement of genotypes within each replication is the degree of randomization. From a statistical viewpoint, randomization of entries is required to obtain a valid estimate of experimental error. To fulfill the requirement, each entry must have an equal chance of being assigned to any plot in a replication and an independent randomization is required for each replication.

Plant breeders understand the importance of randomization and consider it the ideal procedure for comparison of genotypes. They know that any experiment designed to estimate components of variance must be randomized. There are circumstances, however, in which plant breeders do not use complete randomization for the comparison of genotypes. Genotypes with similar characteristics may be planted next to each other to reduce interplot competition in unbordered plots. A nonrandom arrangement of genotypes among replications may be used to facilitate selection of genotypes before harvest.

Nonrandom Arrangements of Genotypes. Any discussion of nonrandom arrangements of genotypes can be misinterpreted because it may imply that randomization is not an important principle. To avoid such misinterpretation, it should be stated again that nonrandomization should only be considered when resources are not adequate to make randomization feasible. The discussion of nonrandom arrangements will include the reasons for their use, their disadvantages, and the ways procedures can be modified to permit effective randomization.

Nonrandomization Among Replications. It is common to delay replicated tests for yield until genotypes have been visually selected in unreplicated plots for characteristics such as lodging, height, and maturity. To reduce the length of time for cultivar development, the season for evaluation in unreplicated plots can be eliminated by growing genotypes in replicated plots, visually selecting those with desirable characteristics, and harvesting only the plots of selected genotypes for yield evaluation (Garland and Fehr, 1981). When visual selection is based on the performance of genotypes in all of the replications, it is necessary to evaluate each plot, summarize the data, make the selections, and identify the plots of selected genotypes that should be harvested. The length of time between plot evaluation and harvest may be only a few days when characteristics of interest are not expressed until plant maturity. If several thousand genotypes are randomized in two or more replications, summarization of data and identification of plots to be harvested can be difficult or impossible to accomplish in only a few days. The use of the same arrangement of genotypes in each replication makes the job practical.

When genotypes are in the same position within each replication, the data for plots of each genotype are recorded in adjacent columns (Fig. 19-7). Sum-

Nonrandom

		Replication		
Plot	Entry	1	2	3
1	1			
2	2			
3	3			
4	4			
5	5			
6	6			

Random

		Replication
Plot	Entry	1
1	4	
2	1	
3	6	
4	3	
5	5	
6	2	

		Replication
Plot	Entry	2
1	5	
2	4	
3	2	
4	1	
5	6	
6	3	

		Replication
Plot	Entry	3
1	2	
2	6	
3	3	
4	5	
5	1	
6	4	

Figure 19-7 Field book pages for recording the data of genotypes grown in three replications. Nonrandom arrangement of genotypes involves one page, whereas a random arrangement involves three separate sections on one or more pages.

marization of data is complete as soon as the last plot is rated. Genotypes with undesirable characteristics in one or more replications can be identified and discarded. The plots of desirable genotypes are readily identified for harvest because they are in the same position in each replication.

The disadvantages of nonrandomization relate to the fact that the same genotypes are always adjacent to each other, which can have negative effects on the comparison of genotypes.

1. In unbordered plots, intergenotypic competition can bias the performance of genotypes more seriously in a nonrandom than in a random arrangement. When a poor competitor is bordered by a good competitor, yield of the poor competitor can be reduced and that of the good competitor increased in every replication. There is no opportunity for a genotype to occur next to others with a more similar competitive ability.
2. In unbordered plots, a genotype that dies or is unusually weak in all replications can prevent the accurate evaluation of adjacent genotypes. The performance of adjacent genotypes would never be tested in replications where they were next to healthy genotypes.
3. No unbiased estimate of experimental error can be obtained.

The need to use nonrandomization of genotypes among replications can be avoided by improving the efficiency of procedures for data summarization and evaluation. An efficient procedure would include the use of a computer. Data would have to be entered rapidly into the computer, possibly by entering plot data into an electronic recorder in the field and electronically transferring the information to the computer. Computer programs would be needed to summarize the data and make selections on the basis of standards established by the breeder. Plot identification information for selected genotypes would have to be provided for harvest.

Grouping Similar Genotypes Within Replications. The evaluation of genotypes in unbordered plots can be hampered by bias from intergenotypic competition. Plant characteristics that often contribute to intergenotypic competition in a crop include such factors as differences in height and time of maturity. To reduce intergenotypic competition, genotypes with similar characteristics may be grouped within replications. The position of each genotype may be varied from one replication to the next. This procedure, sometimes referred to as restricted randomization, has the advantage of reducing the effects of intergenotypic competition in unbordered plots. The primary disadvantage is that all genotypes in a test cannot be compared with the same level of confidence. Genotypes within a group are spaced closer to each other than genotypes in different groups and are less affected by environmental variation among plots.

The use of bordered plots eliminates the need for grouping genotypes. The performance of genotypes in plots is not influenced by intergenotypic compe-

tition; therefore, randomization is practical. An increase in land, seed, and other resources will be needed for replacement of unbordered plots with bordered ones.

Experimental Designs for Replicated Tests. The arrangement of genotypes in replicated tests involves primarily the use of either the randomized complete-block design or incomplete-block designs. The Latin square is used only in special circumstances when the number of entries is small (Cochran and Cox, 1957). The honeycomb design can be used for replicated testing but is considered too difficult to implement for a large number of lines (Fasoulas, 1981).

The differences between the randomized complete-block and incomplete-block designs relate to their ability to account for environmental variation within a replication. The two types of design differ in restrictions on the size of a replication, randomization procedures, analysis of data, and comparisons among genotypes.

The terms complete-block and incomplete-block refer to the arrangement of genotypes in an experiment (Fig. 19-8). A block and a replication are equivalent in a randomized complete-block design. A block contains all of the genotypes in the test and is considered complete. Genotypes are divided into more than one block within each replication of an incomplete-block design. The blocks are considered incomplete because they contain only part of the genotypes. A number of different types of incomplete-block designs are available (Cochran and Cox, 1957). The most common types used in plant breeding are referred to as lattices. In a lattice design, a replication is divided into blocks that collectively contain all the genotypes in a test (Fig. 19-8).

The incomplete-block designs are intended to provide more control over environmental variation within a replication than is possible with the complete-block design. The ideal situation for genotype evaluation would be to test each genotype in the same plot, thus avoiding any environmental variation caused by differences in soil fertility, moisture, and other factors within a field. This is not possible, so the next best approach is to adjust the performance of each genotype according to the relative productivity of the plot in which it is evaluated. If one plot has better fertility and moisture than the average for all plots in a replication, the performance of a genotype in that plot will be adjusted downward. A genotype in a plot with lower productivity than the average will have its performance adjusted upward.

Although individual plot adjustments are not possible, the lattice designs permit the performance of a genotype to be adjusted upward or downward according to the productivity of the blocks in which it was grown. The randomized complete-block design does not divide the replication into smaller units and is not able to adjust the performance of a genotype for environmental variation within replications.

The effectiveness of the lattice design in accounting for environmental variation within replications depends on the pattern of variation. Figure 19-9 shows two replications with variation in soil productivity. The soil productivity in

Block						
	Replication 1					
1	1	2	3	4	5	6
2	7	8	9	10	11	12
3	13	14	15	16	17	18
4	19	20	21	22	23	24
5	25	26	27	28	29	30
6	31	32	33	34	35	36
7	37	38	39	40	41	42
	Replication 2					
1	7	13	19	25	31	37
2	1	14	20	26	32	38
3	2	8	21	27	33	39
4	3	9	15	28	34	40
5	4	10	16	22	35	41
6	5	11	17	23	29	42
7	6	12	18	24	30	36
	Replication 3					
1	12	17	22	28	33	38
2	2	13	24	29	35	40
3	4	9	20	25	36	42
4	6	11	16	27	32	37
5	1	7	18	23	34	39
6	3	8	14	19	30	41
7	5	10	15	21	26	31

Figure 19-8 Lattice design for an experiment with 42 entries and three replications. (Adapted from Cochran and Cox, 1957.) For a randomized complete-block design, there are no blocks within a replication and the entries are assigned at random to the 42 plots.

replication 1 increases from left to right. The blocks of the lattice design are arranged in a pattern that effectively measures the variation, as evidenced by differences in the mean for each block. The variation in soil productivity in replication 2 does not fit a consistent pattern. Much of the variation occurs within blocks, and the mean performance of the blocks is relatively similar. The lattice

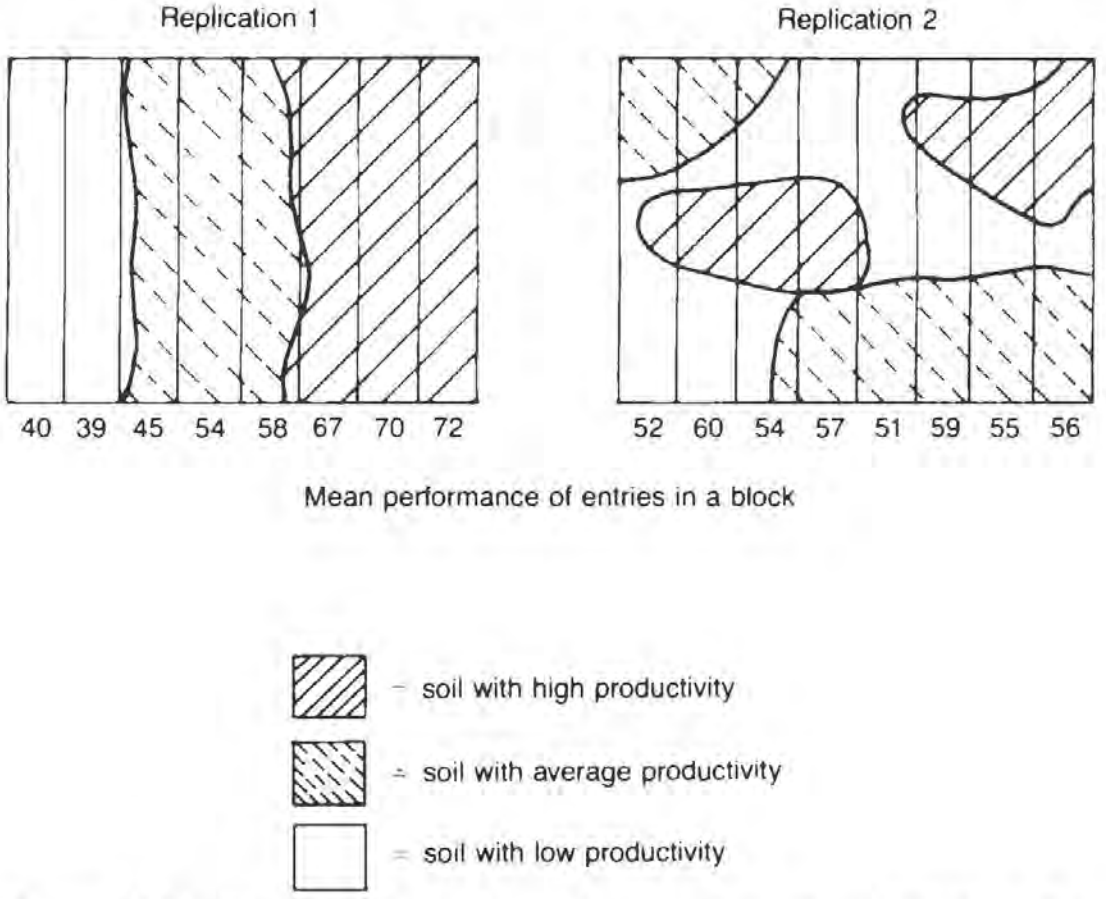


Figure 19-9 The effect of the pattern of variation in soil productivity on the effectiveness of the lattice design in accounting for environmental variation within a replication. The lattice would be more effective in replication 1 than in replication 2.

design cannot adjust for differences in productivity within a block; therefore, it would not be as effective in replication 2 as in replication 1.

The effectiveness of the lattice design compared with the randomized complete-block is expressed as relative efficiency. Relative efficiency is computed as a ratio of mean squares for experimental error of the two types of design.

$$\text{Relative efficiency} = \frac{\text{mean square for error of lattice}}{\text{mean square for error of randomized complete-block}} \times 100$$

The ratio is used to determine the number of replications that would have to be used with the randomized complete block to achieve a precision in detecting differences among the means of genotypes equal to that with a lattice design. A relative efficiency of 150 percent indicates that 50 percent more replication would have been needed with a randomized complete-block design than with a lattice.

The two types of design differ in the flexibility that is possible in a test. The randomized complete-block can accommodate any number of genotypes or replications. The lattice design requires that a specified number of genotypes and replications be included. For example, no lattice design can be used with 44, 58, or 74 genotypes. There is no restriction in a randomized complete-block for the length and width of a replication. For example, a test with 72 entries could be planted 8 plots long by 9 plots wide or 6 plots long by 12 plots wide. The shape of replication for a particular number of genotypes in a lattice is not as flexible. A test with 72 entries could be planted 8 plots long by 9 plots wide, not 6 plots long by 12 plots wide.

The randomization of an experiment and statistical analysis of data are more complex for a lattice than for a randomized complete-block. This can be important if the work is done by hand, but not if done by computer. Computer programs are available that will readily accommodate either type of design.

EQUIPMENT FOR EFFICIENT EVALUATION OF GENOTYPES

The efficient evaluation of a large number of genotypes is important for genetic improvement. Plant breeders have been actively involved in the development of equipment that permits them to evaluate more genotypes with equal or greater quality than was previously possible. The equipment ranges from simple hand devices to sophisticated computers.

Each crop has unique characteristics that influence the type of equipment used. Even for a certain crop, breeders differ as to the type of equipment they consider most desirable. Here only a small sample of available equipment will be used to illustrate how large numbers of genotypes are evaluated by plant breeders.

Preparation of Seed for Planting

The main steps involved in preparing a field experiment include packaging the seed and placing it in the proper arrangement for planting. Computers can be used to randomize entries and assign plot numbers. The computer system can print an adhesive label for each packet of seed to be packaged. The label contains the plot number, the entry number, and other information of value to the breeder. The plot and entry information also can be printed on pages used to record data in the field. The same work can be done by hand, but would require a large amount of labor and would be more subject to human error.

Seed is counted by hand or by electronic counting devices. If the number of seeds for a plot is large and precise numbers are not required, the seeds may be measured by volume.

Planting

Rapid planting of plots can be accomplished with engine-driven planters. Multiple-row plots may be planted from a single packet when each row does not require the exact same number of seeds. The seed is passed through a divider that separates the seed into a fraction for each row. The divider may be a powered spinning device or a gravity system.

The planter can move through the field without stopping. Seed for a row is placed in a container above a planting cone. When the row is to be planted, the container is lifted and the seed drops onto the planting cone. Two types of cones are used to distribute seed along the row. For one type, the base turns and carries the seed to the outlet. There it is knocked from the base by a stationary plate, falling through the outlet to the soil. This type of cone is used for relatively small seeds that do not roll easily, such as barley. The second type has fins mounted on the center cone. The seed falls onto a stationary base and is dragged by the fins to the outlet. The fins are well suited to relatively large seeds, particularly those that have a tendency to roll easily, such as maize and soybean. The length of a plot is a function of the distance traveled by the planter before all the seed has left the cone. At a constant ground speed, a cone must turn faster for short rows than for long rows. Adjustment of the speed of the cone rotation can be accomplished readily by several mechanical systems.

While the seed for one plot is being planted, the seed for the next plot is put in the container above the cone. There are a number of ways to determine when the container should be lifted to begin a plot. One way is to mark the beginning and end of each plot in the field before planting starts. When the planter reaches the beginning of a plot, the operator lifts the containers manually or electronically. The advantage of this procedure is that the location of each plot can be identified as soon as planting is complete. The second way is to use a cable extended across the field that has knobs spaced along it. The spacing between knobs is equal to the length of the plot and the alley. For plots that have rows 5 m long with a 1 m alley between them, the knobs would be spaced 6 m apart. As the planter passes by the cable, the knobs signal when the container should be lifted manually, or it activates an electronic tripping device. The cable is moved after each pass across the field. Use of the cable saves time at planting by eliminating the need to mark the start and end of plots manually.

Weed Control

Weed control is accomplished by the use of chemicals, cultivation, and hand weeding. The chemicals generally are those applied for weed control in commercial production of the crop. Cultivation equipment may be especially designed for use in research fields or may be the same equipment used commercially.

Preparation of Plots for Harvest

Trimming of plots to a constant length before harvest is done manually or with specialized equipment. Plots of small grains generally are trimmed to a constant length early in the season when the plants are about 30 cm tall. A rototiller or mower is passed along the end of each plot to kill the unwanted plants. The rototiller may be mounted on a tractor or may be a self-propelled unit that a person walks behind. Plots of soybean can be cut to a constant length with rotary mowers before seed filling begins. Two mowers are attached to a pipe so that they are separated by a distance equal to the desired plot length, and are driven perpendicular to the length of the rows.

Harvest

The most common type of harvester for the measurement of forage yield in the United States is a self-propelled flail chopper. The machine cuts the plants with a rotating flail that throws the cut portion into a collection point behind the driver. The plant material for a plot may be collected in a plastic container and weighed on a stationary scale set up in the field. To eliminate the labor required to use containers, an electronic scale can be mounted on the machine. The plant material is weighed and then it is discarded into a wagon.

The harvest of plots for their seeds is conducted with three different procedures or types of equipment. One procedure is to collect that part of the plant that bears the seed, weigh it directly, or carry it to a stationary machine for threshing. The plant part may be removed by hand or may be collected with a machine, such as a mower with a collection basket mounted behind the sickle. The harvested sample may be threshed immediately or dried for a period of time before threshing. One popular type of stationary machine is the Vogel thresher. The plants pass vertically through the machine as they are threshed. For a second type of stationary thresher, the material passes through the threshing cylinder and falls on a sieve that helps separate the seed from the plant debris. Air is used to separate the seed and the plant debris in both types of machine.

The second procedure for harvesting plots is to use a self-propelled thresher specifically designed for small plots. The plant part with the seed is gathered into the machine and passes through a threshing cylinder, then the seed and plant debris are separated by sieves and air. The seed may be placed into a bag and saved or may be weighed immediately and discarded. Seed harvested from self-propelled machines generally is more subject to mixtures than that harvested with a stationary thresher.

The third type of equipment is a commercial combine modified for the harvest of small plots. A commercial unit is used only when the amount of seed harvested

from a plot is relatively large and is not saved for planting. Modifications of the commercial combine include reduction of the number of rows harvested and the addition of equipment for weighing the seed.

Data Collection

Usually a number of characters are measured on each plot, such as height, standability, and yield. The data may be recorded in a field book, then manually entered into the computer for statistical analysis. Alternatively, the information may be recorded in an electronic data collector and transferred directly to the computer. This saves time and reduces the possibility of human error. Plot and entry designations also can be recorded on labels that can be read into the data collector by an electronic scanner.

Data Analysis

Computers facilitate the selection of lines by summarizing data in whatever manner is beneficial to the breeder. They save an extensive amount of time, minimize human error, and permit data to be summarized in a short period of time.

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CHAPTER TWENTY

Mutation Breeding

Genetic variability is required to improve a crop for any character. Mutations that occur spontaneously or are artificially induced can be useful sources of variability for the plant breeder.

Mutation breeding has been used to develop improved cultivars of many crops. Sigurbjornsson and Micke (1974) and Sigurbjornsson (1983) reviewed cultivars that had been developed by mutation breeding. The crops and ornamentals they included were bread wheat, durum wheat, barley, oat, rice, soybean, string bean, French bean, navy pea bean, haricot bean, pea, lupine, subterranean clover, seradella, lespedeza, red clover, Italian ryegrass, peppermint, lettuce, tomato, potato, onion, spinach, spring rape, white mustard, castor bean, tobacco, cotton, peanut, peach, cherry, apricot, citrus, apple, carnation, chrysanthemum, dahlia, achimanes, streptocarpus, alstroemeria, rose, and azalea. The characters they cited for which crop cultivars had been improved included yield, lodging resistance, disease resistance, maturity, stem length, food quality, winter hardiness, protein content, shattering resistance, plant type, ease of harvesting, morphology, grain color, seed weight, sprouting resistance, drought resistance, lysine content, and adaptability. It is clear that artificial mutation can be a practical means of obtaining genetic improvement in crop species.

Although cultivars have been developed by mutation breeding, the number is extremely small when compared with the number of those developed by hybridization and selection. Extensive studies of the use of artificial mutagenesis for genetic improvement of crop species during the 1950s and 1960s demonstrated that mutation breeding was of limited value for improving characters of economic value, particularly those that are quantitatively inherited. The relative lack of success of mutation breeding could lead to the conclusion that it has no place in a modern plant breeding program. A more appropriate conclusion, however,

is that the role of mutation breeding for genetic improvement of a crop species should be carefully defined.

It is generally agreed that mutation breeding is most appropriate when a desired character is not available in the germplasm that can be used for hybridization and selection. The frequency of a desired genetic change from artificial mutagenesis generally is low; therefore, the probability of success is greatest when a large number of individuals can be screened for the character.

MUTAGENIC AGENTS

The average rate of spontaneous mutation is approximately 10^{-6} for an individual gene. The goal of artificial mutagenesis is to increase the rate of mutation for the desired characteristic. Mutation rates can be increased by many different means, including temperature, long-term seed storage, tissue culture conditions, radiation, and chemical mutagens. At this time, radiation and chemical mutagens are the most effective. The agent selected depends on its availability, mode of action, and effectiveness for achieving the desired genetic change.

Radiation

Radiation is produced by high energy particles disrupting chemical bonds and transforming one compound into another. Several types of radiation are available (Table 20-1).

X-Rays. X-rays are a commonly used type of radiation for mutagenesis. The treatment can be applied with an X-ray machine capable of producing radiation with the desired wavelength. X-rays with short wavelengths (hard X-rays) have greater penetration but have less potential for creating molecular changes than those with longer wavelengths.

X-rays are electromagnetic radiations created by electrically accelerating electrons in a high vacuum, then decelerating them by having them strike a target, such as molybdenum or tungsten. The abrupt stop of the electron causes the emission of radiation as photons. The photons provide the energy needed to create molecular changes in the cell.

Gamma Rays. Gamma rays are electromagnetic radiations that are produced with the use of radioisotopes and nuclear reactors. Treatments can be given in single doses, or plants can be exposed continuously to gamma radiation over an extended period of time.

The two main sources of gamma rays are cobalt 60 and cesium 137. Because the radioisotopes are dangerous and highly penetrating, they are stored in lead containers and moved by remote control mechanisms to irradiate plant material.

Table 20-1 Properties of Different Types of Radiation Available for Artificial Mutagenesis

Type of Radiation	Source	Description	Hazard	Necessary Shielding	Penetration into Tissue*
X-rays	X-ray machine	Electromagnetic radiation	Dangerous, penetrating	A few millimeters of lead, except for very high-energy installations	A few millimeters to many centimeters
Gamma rays	Radioisotopes and nuclear reactors	Electromagnetic radiation similar to X-rays	Dangerous, very penetrating	Requires very heavy shielding, e.g., centimeters of lead or meters of concrete	Many centimeters
Neutrons (fast, slow, and thermal)	Nuclear reactors (piles), accelerators	Uncharged particle slightly heavier than proton (hydrogen atom), not observable except through its interaction with nuclei in material it traverses	Very hazardous	Thick shielding composed of light elements, such as concrete	Many centimeters
Beta particles, fast electrons, cathode rays	Radioisotopes or accelerators	An electron (+ or −) that ionizes much less densely than alpha particles	May be dangerous	Thick sheet of cardboard	Up to several millimeters
Alpha particles	Radioisotopes	A helium nucleus that ionizes very heavily	Very dangerous internally	Thin sheet of paper	Small fraction of a millimeter
Protons, deuterons†	Nuclear reactors, accelerators	Nucleus of hydrogen	Very hazardous	Many centimeters of water or paraffin	Up to many centimeters

*Penetration depends on many variables, but it is assumed that penetration is into ordinary plant tissue of average density.

†A proton is the nucleus of the common isotope of hydrogen; a deuteron is the nucleus of the heavy isotope of hydrogen.

Source: *Manual on Mutation Breeding*, 1977.

A treatment consisting of a single dose of radiation at a high level is applied in a specially designed facility in a laboratory. For treatment consisting of long-term exposure to gamma rays, plants are grown next to a radioisotope; the distance from the radiation source determines the level of treatment. Specially designed facilities for long-term exposure have been constructed in the field, in the greenhouse, and in controlled-environment chambers in shielded rooms.

Neutrons. The neutrons used for mutagenesis are the product of nuclear fission by uranium 235 in an atomic reactor. In nuclear fission, neutrons with a large amount of energy are produced. Some of the neutrons react with other atoms of uranium to continue the fission process, some are absorbed within the reactor, and some pass from the reactor through outlets. These can be used for mutagenesis. Fast neutrons are those that have high energy levels, as emitted from the reactor. Thermal neutrons containing lower levels of energy are produced by reducing the energy of fast neutrons.

Beta Radiation. Beta electrons are negatively charged particles that are emitted from radioisotopes, such as phosporous 32 and carbon 14. The plant material can be exposed to the radioisotope directly or in solution.

Ultraviolet Radiation. Ultraviolet radiation is used primarily for treating pollen grains because of its low ability to penetrate tissue. Wavelengths from 2500 to 2900 nm are most effective, because nucleic acids have maximum light absorption in that range.

Chemical Mutagens

There is a wide range of chemical mutagens with varied modes of activity. The *Manual on Mutation Breeding* (1977) divides chemical mutagens into seven groups on the basis of the main functional group that determines the type of chemical action (Table 20-2).

Among the large array of available chemical mutagens, the most commonly used are ethyl methanesulfonate (EMS), diethyl sulfate (DES), ethyleneimine (EI), ethyl nitroso urethane (ENV), ethyl nitroso urea (ENH), and methyl nitroso urea (MNH).

TYPES OF MUTATIONS

The four types of mutations that can occur are genome mutations, structural changes in the chromosomes, gene mutations, and extranuclear mutations (*Manual on Mutation Breeding*, 1977). Genome mutations involve changes in the

Table 20-2 Groups of Available Chemical Mutagens

Mutagen Group	Sample Product
Base analogues	5-bromo-uracil, 5-bromo-deoxyuridine, 2-amino-purine
Related compounds	8-ethoxy caffeine, maleic hydrazide
Antibiotics	Azaserine, mitomycin C, streptonigrin, actinomycin D
Alkylating agents	
Sulfur mustards	Ethyl-2-chloroethyl sulfide
Nitrogen mustards	2-chloroethyl-dimethyl amine
Epoxides	Ethylene oxide
Ethyleneimines	Ethyleneimine
Sulfates, sulfonates, sulfones, and lactones	Ethyl methanesulfonate
Diazoalkanes	Diazomethane
Nitroso compounds	N-ethyl-N-nitroso urea
Azide	Sodium azide
Hydroxylamine	Hydroxylamine
Nitrous acid	Nitrous acid
Acridines	Acridine orange

Source: *Manual on Mutation Breeding*, 1977.

chromosome number due to the addition or loss of entire chromosome sets or parts of sets. Structural changes in the chromosome include translocations, inversions, duplications, and deficiencies. (Chaps. 4 and 5).

Mutations that involve a change in a single nucleotide base of a gene commonly are referred to as point mutations. The expression of a gene involves the arrangement of the four nucleotides of DNA in a specific sequence. The expression of the gene can be altered by substituting one nucleotide base for another or by adding or deleting a nucleotide. Gene mutations are commonly associated with use of chemical mutagens, but may occur with radiation treatments. The genetic alteration can cause sterility, can be lethal, or may result in the formation of a new, highly desirable character.

Extranuclear mutations involving components of the cytoplasm are known to occur. Cytoplasmic-genetic male sterility is an example of a cytoplasmic mutation that is useful for crop production. DNA involved in extranuclear mutations occurs in the plastids and mitochondria. Genetic changes in these organelles are transferred from one generation to the next primarily through the egg cells. Ethidium bromide has been used to create cytoplasmic changes in crop species. Additional mutagens that may be considered are acridines, streptomycin, dyes, heavy metal salts, EMS, and radiation treatments.

PLANT MATERIAL TO BE TREATED

Whole Plants

The treatment of seedlings or small plants can be accomplished with X-rays. Gamma rays can be used to treat small or large plants in a gamma field or gamma room.

Seeds

Seed is the most common plant material treated with radiation or chemical mutagens for seed-propagated species. Seeds are generally preferred because they tolerate physical conditions normally tolerated only by nonliving molecules, such as being desiccated, soaked, heated, frozen, or maintained under varying levels of oxygen and other gases.

Pollen Grains

Pollen grains can be treated with radiation or chemical mutagens. The advantage of treating pollen grains is that the zygote and the plant that develops from it are heterozygous for any genetic change that occurs in the pollen (Fig. 20-1). In contrast, the treatment of seed or whole plants can result in chimeras, in which part of the plant differs genetically from another part. A disadvantage of pollen treatment is that an adequate supply of viable pollen can be difficult to obtain and maintain for some species.

Parts of Plants Used for Asexual Propagation

The treatment of cuttings or apical buds with radiation or chemical mutagens can be effective in developing mutant types in new shoots and plantlets. The important factor is to treat the meristematic region from which new propagules develop.

Cell and Tissue Culture Explants

The use of radiation and chemical mutagens in cell and tissue culture is a rapidly expanding field of research. The concept is to treat single cells or tissue explants, screen them on a medium that will identify the mutant type, and regenerate the desired types into whole plants.

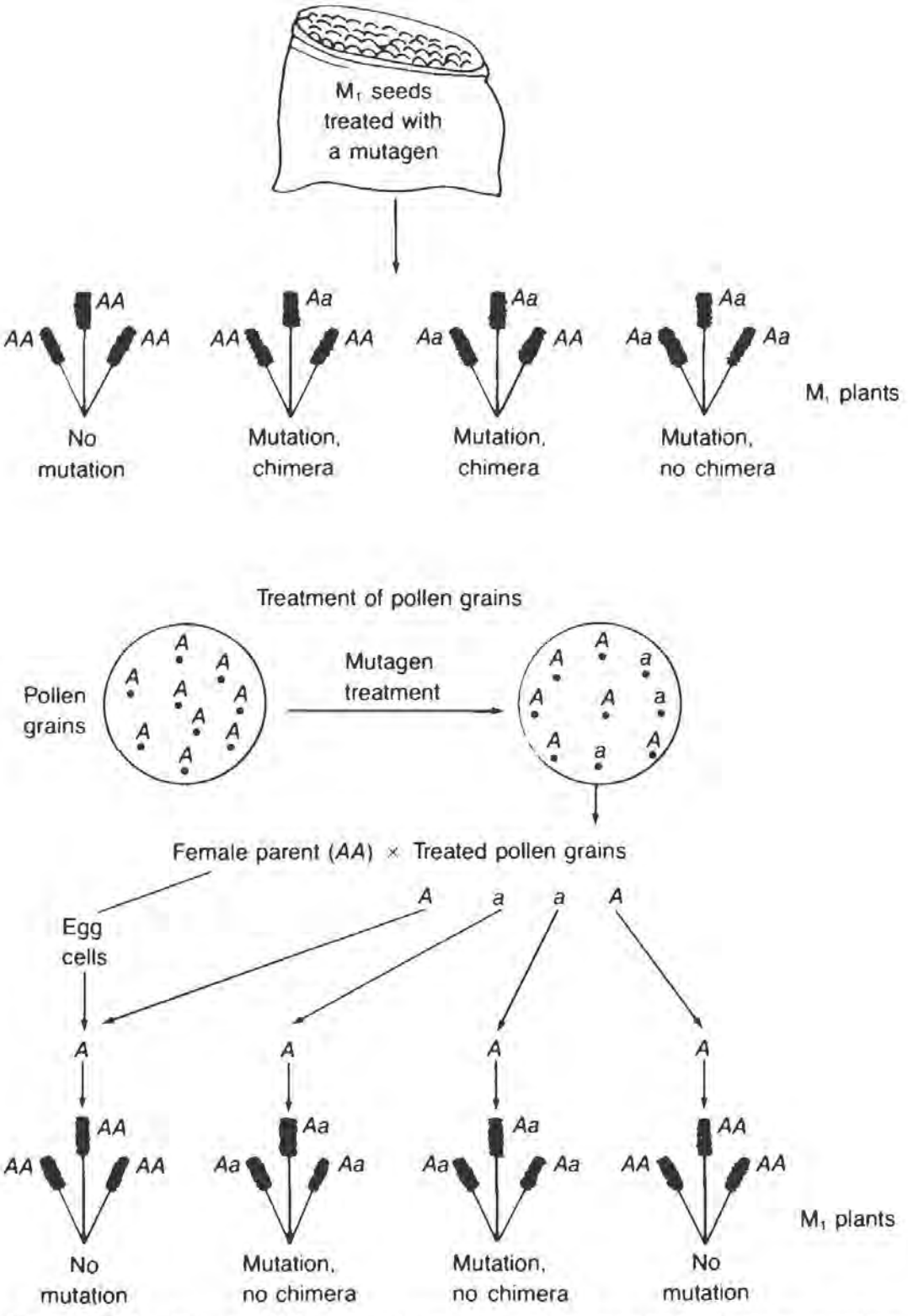


Figure 20-1 Genetic differences between populations of M_1 plants grown from treated seeds and plants obtained from treated pollen grains. The genotype of the untreated seeds is AA and of the untreated pollen grains is A. A mutation is represented by the allele *a*.

FACTORS TO CONSIDER WITH MUTAGEN TREATMENTS

The frequency and type of mutation recovered can vary with the dose and rate at which the mutagen is applied, the species, the genotype within a species, oxygen level, water content, temperature, pretreatment conditioning, treatment conditions, and posttreatment conditions. Each of these variables must be considered in developing a procedure that will be effective.

Dose and Rate

The dose at which a mutagen is applied and the duration of the application are referred to as the dose rate. An acute treatment is one made in a short time, such as a few minutes or hours. A chronic treatment involves exposure to the mutagen for long periods of time, such as weeks, months, or years. The dose rate is a primary variable in a mutagenesis program.

When the desired genetic change involves only a single unit, such as a nucleotide change for a point mutation or loss of part of one chromosome for a deletion, a single exposure to a mutagenic agent for a brief period generally is most effective. When two or more units must be affected simultaneously, such as two chromosomes being broken for a translocation, multiple exposures or chronic exposure generally is preferred.

The appropriate dose rate must be determined by experimentation. For treatment of seeds, the objective generally is to use a dose rate at which 50 percent of the seeds germinate and produce a plant that has viable seed. This is commonly referred to as the LD_{50}.

The most effective way to determine the LD_{50} of seeds is to treat the cultivars of interest with varying dose rates at specified conditions of temperature and moisture, grow the plants in an environment typical of that to be used for later treatments, and count the number of surviving plants. Plant survival for determining the LD_{50} can be estimated from standard germination counts. Such tests can be misleading, however, because plant mortality can occur throughout the growing season. Survival is measured as the ability of a plant to produce at least one viable seed. Germination percentage cannot take into account the number of plants that will be completely sterile. Nevertheless, germination percentage is frequently used to estimate the LD_{50} of chemical mutagens.

Species and Genotypes

There are important differences among species and among genotypes within species for sensitivity to mutagen treatment. Any dose rate study should be conducted on the species that will be used for further mutagen treatment, and preferably on the genotypes to be used.

Oxygen Level

The oxygen level in plant material can influence the amount of damage caused by a mutagen. The higher the oxygen level, the greater the tendency for seedling injury and chromosomal aberrations relative to mutation frequency. The change in mutation effects with oxygen supply is referred to as the oxygen enhancement ratio. To reduce the effect of oxygen, seeds can be treated at high moisture levels or in an oxygen-free atmosphere. In cases where enhanced mutation frequency is desirable, the oxygen effect can be increased by use of dry seeds or by bubbling of air through a solution. For chemical mutagens, the latter is a common practice.

Water Content

The effect of water content is directly related to oxygen supply. High moisture levels reduce oxygen supply, and low moisture levels increase the oxygen supply. Some species are extremely sensitive to changes in water content. Barley seeds at 10.7 percent moisture had a threefold greater response to postirradiation oxygen than seeds at 11.0 percent moisture (Conger et. al., 1968). The effect of water content on mutation frequency is more important for some mutagens than for others. Water content is important in the mutation frequency caused by X-rays and gamma rays. Neither water content nor oxygen supply is crucial when seeds are treated with fast neutrons (*Manual on Mutation Breeding*, 1977).

Temperature

Temperature does not seem to be a crucial factor in radiation treatment but is critical in chemical mutagenesis. The main effect of temperature is on the length of time (half-life) that a chemical is reactive with the plant material. The half-life of a chemical mutagen is the time after which one-half of the initial concentration of the mutagen has reacted. For EMS, the half-life at 40°C is 7.9 hours and at 5°C is 796 hours (*Manual on Mutation Breeding*, 1977). For sulfur mustard, the half-life at 37°C is only about 3 minutes. It is important to know the half-life of a chemical before determining the appropriate treatment procedure.

Pretreatment Conditioning

Adjustment of moisture content to the desired level can be an important part of preparing seeds for radiation and chemical treatments.

Soaking seeds in water is a commonly used pretreatment for chemical mu-

tagenesis. Presoaking leaches out water-soluble substances and hydrates the cell membrane and macromolecules. At temperatures adequate for germination, presoaking can initiate metabolism and DNA synthesis.

When it is preferable to avoid initiation of metabolic activity during presoaking, seeds should be immersed in water at 0°C. It is desirable to keep the water moving by shaking or by having a continuous water flow. The water should be changed every 15 or 30 minutes.

When initiation of metabolic activity during presoaking is desired, the procedure should be the same as just described except that the temperature of the water should be raised and air bubbled through the solution may be desirable. The necessary water temperature and duration of presoaking can be determined by experimentation in which seeds presoaked at different temperatures and durations are exposed to a single dose of a mutagen. The germination percentage and growth of the seedlings will identify the most appropriate treatment.

Treatment Conditions

Temperature of the treatment solution will influence the duration that a chemical mutagen is effective. The pH of the treatment solution can influence the amount of physiological damage and the relative frequency of gene mutations and chromosomal aberrations. There are important differences among chemicals for the pH that is most effective. EMS is commonly used at pH 7.0, whereas sodium azide is most effective at a pH of 3.0. Phosphate buffer of 0.1 M strength or less is recommended if buffers are used in the treatment solution. The use of metal ions in the treatment solution for chemical mutagens is not recommended.

Posttreatment Handling

The handling of treated seeds before planting can markedly influence the amount of survival. Seeds treated with X-rays, gamma rays, or fast neutrons should not be stored more than a few weeks. Oxygen should be eliminated, either by maintaining the seeds at a high moisture level or by placing them in an oxygen-free container. Normal laboratory temperature can be used for storage, unless the seeds are to be kept for extended periods. Seeds should be held at 0°C or less for long-term storage.

With chemical mutagens, posttreatment washing of seeds is recommended to remove residual chemical. Postwashing for 8 hours or more is particularly important if the seeds are to be dried before being planted.

There is a greater rate of survival if moist seeds treated with a chemical

mutagen are planted immediately in moist soil after being washed than if they are dried and stored before planting. No drying procedure has been found that eliminates this phenomenon. If dried seeds must be stored, particularly for extended periods, they should be kept at 0°C or less.

PRECAUTIONS IN THE USE OF MUTAGENS

A mutagenic agent can adversely affect persons who come in contact with it. It is impossible to overemphasize the importance of appropriate safety procedures in using any of the mutagens that are available. The importance of appropriate safety precautions for use of the different types of radiation seems to be well understood. Furthermore, the facilities required for radiation treatments are available in only a limited number of locations and are operated by trained personnel.

Appropriate handling of chemical mutagens is as essential as for radiation treatments. To use chemical mutagens safely, personnel must be aware of the procedures involved in safe storage, handling, and disposal of a chemical mutagen. Many chemical mutagens undergo dangerous reactions upon contact with certain compounds. Gloves, lab coats, safety glasses, pipette fillers, and other equipment should be used to avoid personal contact with mutagens. Procedures and equipment should be readily available to contain and clean up accidental spills in the laboratory or provide medical assistance in the case of human contact with a mutagenic chemical. The *Manual on Mutation Breeding* (1977) describes physiochemical properties, appropriate storage, cleanup and disposal procedures; dangerous reactions; and health hazards of various classes of mutagenic compounds.

BREEDING PROCEDURES FOR SEED-PROPAGATED SPECIES

The plant breeder must make a number of important decisions with regard to development of populations by artificial mutagenesis and selection of mutants within the populations.

Objective of the Mutation Breeding Program

The characters to be changed by mutation breeding should be clearly defined. Selection for one character will have a greater chance of success than selection for two or more characters simultaneously. Efficient methods of screening large numbers of plants should be developed to increase the chance of finding the desired mutant type.

Selection of the Parents

Selection of an appropriate parent depends on the objective of the mutation program. If the objective is to release a cultivar with improved characteristics, the parents should be existing cultivars or experimental lines with favorable characteristics for all traits except the one(s) to be changed. Selection of a parent for such a breeding program is essentially the same as choice of a recurrent parent in a backcrossing program.

When mutation breeding is used to create a characteristic that does not occur in the species, a breeder has two choices in selecting parents. The parent can be an agronomically desirable cultivar or experimental line that is inferior for the characteristic. Alternatively, a parent can be chosen that has the best level of the character, regardless of its other characteristics. For example, mutation breeding is being used in an attempt to lower the linolenic acid content of soybean oil to less than 3 percent. The best adapted cultivars have about 8 percent linolenic acid. Plant introductions with poor agronomic characteristics are available with 5 percent linolenic acid. Use of adapted cultivars provides a chance for release of a cultivar directly if an appropriate mutant type is found, but the amount of genetic change needed to reach the desired objective is greater than for the plant introductions. Use of the plant introductions requires less of a genetic change to meet the goal; however, any desirable mutant types could not be released as a cultivar directly. Whichever type of parent is chosen, several different genotypes should be treated to compensate for possible genetic differences in mutability.

Seed Source of the Parent Cultivars

When mutation breeding is used to improve a characteristic of a cultivar, the seed treated should be representative of the cultivar. A homogeneous supply of a crop, such as from breeder or foundation seed, is desirable to avoid confusion over which off-types are mutants and which are the result of seed mixtures or hybridization. For cross-pollinated populations, the seed source will be heterogeneous and heterozygous; therefore, differentiation between mutants and segregation products may be difficult.

Seed Treatment

Selection of an appropriate dose and rate can have an important influence on the success of artificial mutagenesis. When information is limited as to the type of mutagen to use for a crop species, it is advisable to use two or more different mutagens with several doses of each. A control of untreated seeds should be grown with each generation of inbreeding to provide a comparison for evaluation

of treatment effects. The control seeds should be handled the same as the treated seeds through all procedures, except that they are not exposed to the mutagen. For example, the handling of controls for chemical mutagens should include such procedures as presoaking and postwashing of the seed.

Number of Seeds to Be Treated

The number of seeds to be treated must be based on percentage survival of the M_1 plants, the number of plants that are to be evaluated each generation beginning with the M_2, and the method of handling the treated material each generation. Mutation frequency for a character also is desirable information, but often is not known with precision.

Survival of M_1 plants should be predicted on the basis of preliminary experiments. Laboratory and greenhouse tests may be less severe than field conditions; therefore, survival potential may have to be adjusted according to the environment in which the M_1 plants will be grown.

The number of M_2 plants desired and the method of handling the population during self-pollination will determine the number of M_1 plants required. The number of M_2 plants desired is dependent on the expected mutation frequency. If this cannot be predicted, it is necessary to grow as large an M_2 population as resources will permit.

Growing the M_1 Generation

When a large M_1 generation is desired, the seeds generally must be planted in the field. Survival of the M_1 plants can be influenced by the care taken in planting the seed and in maintaining the planting.

Isolation of M_1 plants from other genotypes of the same species may be important if plants are not to be self-pollinated by hand. For naturally cross-pollinated species, artificial self-pollination is required to obtain M_2 seeds. For self-pollinated species, however, manual self-pollination generally is not conducted and isolation may be important. Mutagen treatments commonly cause some degree of pollen sterility, making self-pollinated species more susceptible to outcrossing. Outcrossing is a problem if it is important to distinguish between character changes caused by mutation and those caused by accidental cross-pollination. Isolation can be accomplished by planting the genotypes an adequate distance apart, by use of different planting dates, by surrounding the genotypes with a different species, or by enclosing the flowers or plants in a bag or cage.

The seeds should be planted at a time conducive to rapid germination and emergence. Unfavorable environmental conditions can inhibit the survival of seedlings weakened by the mutagen treatment. The soil should be friable, moist, properly fertilized to promote plant growth, and at a favorable temperature.

Dry seeds often can be planted mechanically. Wet seeds treated with a chemical mutagen must be planted by hand into moist soil. Rubber gloves should be worn to avoid contact with any residual mutagen on the seed. The soil should be kept moist until the seedlings emerge.

Some species are more easily germinated in the laboratory and transplanted to the field. No selection for vigor of plants should be practiced in choosing M_1 plants for transplanting.

It has been found that mutations are more likely to be present on the main stem than on tillers and branches. The plant density should be high enough to prevent tillering and branching and still permit differentiation of individual plants.

Herbicides can be used for weed control, but they must be nonsystemic ones. A systemic herbicide may influence the vigor and survival of the plants.

In addition to the notes normally taken on breeding plots, additional data on M_1 plants often are desirable. Determination of the percentage emergence of treated seeds, seedling survival, and plant survival to maturity is important in identifying an appropriate mutagen and a proper level of treatment. Notes on the sterility of M_1 plants can provide information on the type of genetic alterations induced by the mutagen and on appropriate procedures for generation advance. Sterility can be estimated from observations made at the time of flowering or through its effect on seed yield.

Breeding Methods from the M_1 to Later Generations

The challenge for the breeder is to handle the M_1 and later selfing generations in a manner that will maintain and identify any desirable mutations that occur. For a mutation to be recovered, (a) it must occur in a cell that gives rise to the reproductive organs from which a seed is produced, (b) the seed containing a mutation must be harvested from the M_1 plants and planted as part of the M_2 and later generations, and (c) any recessive mutation must be homozygous for its phenotype to be expressed.

When a mutation occurs in a pollen grain that fertilizes an egg, all cells of the seed and the plant that develops from it will bear the mutation in the heterozygous condition (Fig. 20-1). Treatment of seed, however, usually causes a genetic change in only part of the meristematic cells. As a result, the plant may have parts that differ genetically, referred to as a chimera. For example, the main stem of a plant may have developed from a mutated cell, while all the tillers or branches developed from an unmutated cell in the seed. If seeds are harvested only from the tillers or branches of the plant, the mutation will not be recovered. The method used to handle the M_1 and later generations, therefore, can influence recovery of a mutation.

The breeding methods that can be used during the selfing generations are the same as those available for a population developed by hybridization. The M_1 and F_1 generations are genetically similar in the sense that plants can be heterozygous at a locus. Populations of M_1 and F_1 plants are quite different, how-

ever, because the M_1 plants in a population can be different genetically, some possessing a specific mutation and others not. All F_1 plants are genetically the same if they were derived from a cross between two inbred parents. A population of M_1 plants must be handled as if it were segregating as an F_2 population.

Each of the breeding methods has advantages and disadvantages for maintaining and selecting mutant types. With a fixed amount of resources for testing M_2 plants, a choice must be made between evaluating multiple progeny from a smaller number of M_1 plants or one or a few progeny from more M_1 plants.

Pedigree Method The steps in the pedigree method for inbreeding a population by artificial mutagenesis are

Season 1: M_1 plants are grown and harvested individually.

Season 2: Sufficient M_2 progeny are grown from each M_1 plant to provide a chance of recovery of a mutant segregate. The method for determining the appropriate number of progeny to obtain for a given probability of success is the same as that described for the backcross method (Chap. 28). M_2 plants that have or seem to have the desired phenotype are harvested individually.

Season 3: M_3 progeny from selected M_2 plants ($M_{2,3}$ lines) are grown. Progeny rows having the desired mutant phenotype and uniformity for other characters may be harvested in bulk. If a row has the desired mutant phenotype but is segregating for other characters, M_3 plants may be harvested individually. The plants may be progeny tested in season 4 and additional selection among and within rows may be conducted in subsequent generations.

Season 4: The performance of uniform $M_{2,4}$ lines may be evaluated in replicated tests for the character under selection and for other important agronomic characteristics.

Season 5 + : In subsequent seasons, desirable lines may be tested for release as cultivars or for use as parents.

The advantage of the pedigree method is that growing progeny from each M_1 plant provides a good opportunity for recovering a mutant if it was present in the M_1 individual. The number of M_1 plants that can be evaluated, however, would be less than in the case of single-seed descent. The labor required for pedigree selection is greater than that needed for the other methods.

Bulk Method The maintenance of a population in bulk requires less labor than any other method:

Season 1: M_1 plants are grown and their M_2 seeds are harvested together as one bulk population.

Season 2: A sample of the M_2 seeds from season 1 are planted. Individual M_2 plants may be harvested for progeny testing in season 3 or all of the

plants can be harvested together in bulk. Mass selection may be practiced on the plants before they are harvested in bulk or on the bulk seed.

Season 3: Two procedures are possible: (a) The M_3 progeny from individual M_2 plants harvested in season 2 may be grown. Subsequent selection among and within progeny may be conducted in the same manner as described for pedigree selection. (b) A bulk of M_3 seeds from season 2 may be planted. Individual M_3 plants may be harvested for progeny testing or alternatively, the plants may be harvested in bulk with or without mass selection of the plants or seeds. The bulk procedure may continue for as many generations as desired.

Season 4+: After a desirable line is recovered, it may be used as a cultivar or parent.

With the bulk method, inexpensive forms of mass selection may be useful in the M_2 and later generations to recover the desired mutation. The primary disadvantage of harvesting M_1 plants in bulk is related to the possibility that the individuals containing a high frequency of mutations, including the desired one, may produce fewer seeds than individuals with no mutations. Thus, when a sample of seed is taken from the bulk to plant the M_2 generation, the chance that the seed comes from an M_1 plant with low productivity is less than the chance that it comes from a highly productive individual.

Single-Gene Descent Productivity of an individual does not influence single-seed descent as long as one or a few seeds are available from each plant. When all of the seed harvested from the M_1 populations is planted in the M_2, each M_1 individual can be represented equally in the M_2, except for differences in seed viability.

Season 1: M_1 plants are grown and one or a few M_2 seeds are harvested from each plant and bulked.

Season 2: The M_2 seeds are planted. Desirable M_2 plants may be harvested individually for progeny testing in season 3 or one or a few seeds may be harvested from each plant and bulked. The seeds may be harvested from only those plants with a desirable phenotype.

Season 3: Two procedures are possible: (a) The M_3 progeny from individual M_2 plants harvested in season 2 may be grown. Subsequent selection among and within progeny may be conducted in the same manner as described for pedigree selection. (b) The bulk of M_3 seeds from the single-seed harvest in season 2 may be planted and individual M_3 plants may be harvested for progeny testing. As an alternative, one or a few seeds may be harvested from each plant and bulked. The single-seed descent procedure may continue for as many generations as desired.

Season 4+: After a desirable line is recovered, it may be used as a cultivar or a parent.

An advantage of single-seed descent is that more M_1 plants can be utilized than with the other methods because only a few progeny are sampled from each individual. This also can be a disadvantage, because by chance none of the few seeds sampled may have the mutation, even though it was present in the heterozygous condition in an M_1 plant.

Early-Generation Testing The primary use of early-generation testing would be for quantitative characters that cannot be selected visually:

Season 1: M_1 plants are grown and harvested individually.
Season 2: $M_{1:2}$ lines are grown in replicated plots. The lines with the desired performance are retained.
Season 3: Selected $M_{1:3}$ lines are grown and individual M_3 plants with desirable characteristics are harvested individually.
Season 4: $M_{3:4}$ lines are evaluated in replicated plots.
Season 5+: A line with the desired characteristics may be released as a cultivar or used as a parent.

When lines derived from plants in the M_1 or later generations are evaluated in replicated tests, the assumption is that segregation for the desired mutation will influence performance sufficiently so that the heterogeneous mutated line will be chosen. Selection within the heterogeneous line at the desired level of inbreeding would identify the desired mutants in the homozygous condition.

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CHAPTER TWENTY-ONE

Breeding for Pest Resistance

Pest resistance is commonly an important objective in the development of cultivars. Resistant cultivars have been used effectively for the control of diseases, nematodes, and insects. Genetic resistance in plants can be considered a major form of biological control of pests.

The development of resistant cultivars involves consideration of the genetic variability of the pest as well as that of the plant (Hooker, 1983). The resistance of many cultivars has been effective for only a short period of time due to the emergence of new genotypes of the pest to which the cultivars are susceptible. The longevity of different types of genetic resistance is one consideration in breeding for pest control.

TYPES OF GENETIC RESISTANCE

Resistance to pests can be either a qualitative or quantitative character. Resistance controlled effectively by one or a few genes that result in distinct classes of resistant and susceptible plants is considered qualitative. Resistance that displays continuous variation among genotypes is considered quantitative. Resistance that is qualitatively inherited is commonly referred to as specific or vertical resistance. Resistance that is quantitative is referred to as nonspecific, field, general, horizontal, or polygenic resistance. Each of the terms describes some aspect of the nature of genetic resistance in plants as related to the genetic variability present in pests. The terms specific resistance and general resistance will be used in the following discussion.

Specific resistance is associated with the ability of major genes to control specific races (genotypes) of a pest. The individual alleles of a major gene can be readily identified and transferred from one genotype to another. Segregation

for a major gene can be predicted reliably on the basis of the genotype of the parents of a cross. Furthermore, the presence of the allele can be determined by exposing a particular plant or its progeny to a specific race of the pest.

The primary disadvantage of specific resistance is its vulnerability to new races of a pest. When a cultivar with a major gene for resistance is exposed to a genetically variable population of the pest, it is likely to be susceptible to one or more races. These races may occur at low frequencies within the population and cause no measurable damage to the cultivar. Continuous use of the cultivar, however, may result in an increase in the frequency of the races to which it is susceptible, and the pest can then cause economic injury when the cultivar is grown.

General resistance is caused by the expression of alleles for resistance at many loci, each with a minor effect. The advantage of general resistance is its ability to control a broad spectrum of races in a pest population. New races of the pathogen would have difficulty overcoming the presence of alleles for resistance at many loci.

The disadvantage of general resistance is the difficulty of transferring it from one genotype to another. The individual alleles in a parent cannot be identified; therefore, the frequency of desirable individuals in the progeny of a cross cannot be predicted. The probability of transferring all desirable alleles from a resistant to a susceptible genotype is low when a large number of alleles is involved.

GENETIC INTERACTION OF THE PLANT AND PEST

The susceptibility of a plant is determined by the genetic relationship between it and the pest. This relationship was described by Flor (1956) from the study of flax rust. He indicated that for each gene in the host that controls resistance to rust, there is a gene in the pathogen that determines if the pathogen will be avirulent (unable to injure the host) or virulent (able to injure the host). The relationship between the genes of the host and pathogen determines the disease expression of the host. Flor's proposal for the genetic control of host–pest interaction is referred to as the gene-for-gene hypothesis. The concept illustrates the active interaction between the genetic system of the two organisms (Table 21-1).

The gene-for-gene hypothesis has been likened to a set of locks and keys (Browning, 1963). Each locus in the host that conditions resistance to a pathogen is a potential position for a lock. The locks are dominant alleles that prevent the pathogen from becoming established in the host. The pathogen is not effective unless it has a key for each lock in the host or the host lacks a lock. The keys represent recessive alleles for virulence. When a host has the genotype *AABBcc*, the pathogen must have the recessive alleles *aa* and *bb* to be effective (Table 21-1). The alleles at locus c in the pathogen can be dominant or recessive because the host does not have the dominant allele (lock) necessary for resistance.

Table 21-1 Combinations of Dominant Alleles for Resistance in the Plant and Recessive Alleles for Virulence in the Pest that Result in a Susceptible or Resistant Plant Response

Cultivar	Genes for Resistance in Plant	Genes for Virulence in Pest	Plant Response
1	None	Any virulence gene	Susceptible
2	*A*—	None	Resistant
3	*A*—	*aa*	Susceptible
4	*A*—*B*—	*aa*	Resistant
5	*A*—*B*—	*bb*	Resistant
6	*A*—*B*—	*aabb*	Susceptible
7	*A*—*B*—*C*—	*aabb*	Resistant
8	*A*—*B*—*C*—	*aabbcc*	Susceptible

RACES OF PESTS

Genetic variability for host–pest interactions is based on the differential response of plants to isolates of a pest (Stakman and Piemeisal, 1917). Isolates that cause differential responses among host genotypes are referred to as races.

Knowledge of the genetic variability within a pest population is important in breeding for adequate resistance in cultivars. In cereal crops, a regular survey is made of the races in populations of rust.

MECHANISMS FOR DISEASE RESISTANCE

There are three mechanisms by which plants minimize the impact of disease organisms on their development and reproduction. A plant may resist the establishment of the pathogen in its tissue, it may resist the growth and reproduction of the pathogen that becomes established, or it may develop and reproduce well despite the activity of the pathogen. Commonly the first two mechanisms are considered forms of resistance and the last is described as tolerance.

Resistance to Establishment of the Pathogen

The failure of a pathogen to establish itself successfully in a plant has been referred to by a number of terms, each of which describes some characteristic of this type of resistance (Nelson, 1973).

1. *Hypersensitivity*: Infection by the pathogen is prevented by the plant.
2. *Specific resistance*: Specific races of the disease cannot infect the plant.

3. *Nonuniform resistance*: The host prevents the establishment of certain races but not others.
4. *Major gene resistance*: Races of the disease are controlled by major genes in the host.
5. *Vertical resistance*: Host resistance controls one or a limited number of races. Variation in host resistance is largely dependent on cultivar × isolate interactions.

Resistance to an Established Pathogen

The extent of injury caused by a pathogen depends on its ability to spread and reproduce after becoming established in a host. Resistance of a plant to development of the pathogen has been described by the following terms:

1. *Field resistance*: Inoculation of a plant in the laboratory may cause it to show severe injury, but in the field the plant demonstrates almost normal development when the pathogen is present.
2. *General resistance*: The host is able to resist the development of all races of the pathogen.
3. *Nonspecific resistance*: Host resistance is not limited to specific races of the pathogen.
4. *Uniform resistance*: Host resistance is comparable for all races of the pathogen, rather than being good for some races and not good for others.
5. *Minor gene resistance*: Host resistance is controlled by a number of genes, each with a small effect.
6. *Horizontal resistance*: Variation in host resistance is due primarily to differences between cultivars and between isolates, rather than to specific cultivar × isolate interactions.

Tolerance

Tolerance is the ability of a plant to perform well even though it exhibits the symptoms of a susceptible host. A tolerant plant lacks the ability to prevent the establishment of a pathogen or to retard its development after establishment. On the basis of a visual assessment of symptoms, a tolerant plant would be rated as susceptible, just like a nontolerant one. Despite the exhibition of disease symptoms, however, the performance of a tolerant plant would be similar to that observed in plants without infection.

Some persons use the term tolerance interchangeably with field resistance and other terms that apply to development of an established pathogen. Others prefer to exclude tolerance as a form of resistance.

MECHANISMS FOR INSECT RESISTANCE

The three mechanisms that influence the ability of a plant to grow productively in the presence of an insect are nonpreference, antibiosis, and tolerance (Painter, 1951). One or more of these mechanisms can be operative in a cultivar that is considered insect resistant.

Nonpreference

Nonpreference includes insect responses to plant characters that make a cultivar undesirable for use by insects as a site for reproduction, food, shelter, or any combination of the three. The plant characters that influence nonpreference include color, light reflection, type of pubescence, leaf angle, odor, and taste. Yellow-green cultivars of peas are less desirable to the pea aphid than are blue-green ones (Painter, 1951). The aphid of cabbage is attracted most to plants with leaves that reflect low intensities of light. Soybeans without pubescence can be extensively damaged by the potato leafhopper, while those with pubescence seem to be unaffected. Onion thrips are most prevalent on cultivars that have a small angle of separation between the leaves on which the thrips live. The Colorado potato beetle is influenced by the odor of different potato species. Resistance to grasshoppers in maize and sorghum seems to be related to differences in taste.

Antibiosis

Antibiosis is the adverse effect of plant tissue used as food by an insect on the insect's development and reproduction. The adverse effects may include inhibited growth, death, and prolonged time to maturity (Painter, 1951). Antibiosis is considered by some to be the only true form of insect resistance in plants.

Tolerance

A tolerant cultivar is able to grow and reproduce in spite of supporting a population of insects similar to a population that would damage a nontolerant host. A tolerant and nontolerant cultivar would be indistinguishable when rated for the number of insects present, but the tolerant one would be less affected by them. Tolerance does not involve prevention of insect infestation or injury to the plant; therefore, some persons prefer not to include tolerance as a type of resistance.

The general vigor of a plant is associated with its level of tolerance. Single-

cross hybrids of sorghum and maize that exhibited heterosis were more tolerant to chinch bugs than their susceptible inbred parents (Painter, 1951). The ability of a plant to produce new roots, leaves, or stems can influence its tolerance to damage. Strength of stem tissue is associated with the degree of tolerance to insects that bore into the stem.

BREEDING FOR SPECIFIC RESISTANCE

It is common to find major genes that control the prevalent races in a pest population and other genes that control races of minor importance. There are three general strategies for the use of available genes for specific resistance to prevalent and minor races. The strategy that the breeder adopts has a direct bearing on the types of cultivars that are developed and the breeding method used. One strategy is to develop cultivars that possess a single major gene capable of controlling the prevalent pest. A second strategy is to put genes controlling the prevalent and minor races of a pest into individual genotypes that can be mixed together to form a multiline. The third strategy is to place the genes controlling prevalent and minor races into a single cultivar, a process referred to as pyramiding.

Cultivars with Individual Major Genes

The most widely used strategy in breeding for pest resistance is the development of cultivars with an individual major gene to control the prevalent pest races. Cultivars may be developed by selection among the progeny of a segregating population that contains the allele for resistance or by transfer of the allele through backcrossing. The advantage of major gene resistance is the ease with which a single gene can be handled in a breeding program. Its disadvantage is the vulnerability of a single gene to minor races that may become prevalent in the pest population.

Multilines

Seed of genotypes with individual major genes for pest resistance can be mixed together to form a multiline; also referred to as a blend or mixture (Chap. 32). The individual genotypes can be isolines that differ primarily for the major gene they contain. A multiline also can be composed of genotypes that differ for agronomic characteristics as well as for the major genes for pest resistance.

A multiline can provide protection against a broad spectrum of races of a pest. When there is a change in the frequency of races within a pest population,

some genotypes within the multiline are likely to be resistant to whatever race occurs. These resistant plants reduce the spread of the new race to susceptible plants.

Two primary disadvantages of the multiline strategy have prevented it from being widely adopted. (a) Considerable effort is required to transfer a number of major genes into agronomically superior genotypes. (b) If the backcross method is used to develop isolines, the recurrent parent limits the agronomic characteristics of the multiline. During the years when the backcross program is being conducted, a new cultivar may emerge from other selection programs that is superior agronomically to the recurrent parent. The new cultivar may limit the acceptability of the multiline when it is completed.

Pyramiding

For the pyramid strategy, the major genes that would be put into individual lines for a multiline are incorporated into a single cultivar. More major genes may be used than are necessary to give adequate resistance to prevalent races of the pest. The diversity of major genes is expected to provide protection against new races that may develop in the pest population.

The pyramid strategy has several disadvantages that limit its use. (a) Considerable effort is required to incorporate many major genes into one genotype. Extensive testing with different races is required to ensure that each of the desirable alleles is present. (b) Use of the backcross method to incorporate the major genes into one genotype restricts the agronomic characteristics of the new cultivar to those of the recurrent parent. (c) Resistance of the cultivar may encourage the evolution of new virulent races of the pest, particularly if the same major genes are used singly in other cultivars.

MINIMIZING CHANGES IN RACES

Changes in the prevalent races of a pest population are a major concern in crop production. A crop is vulnerable to major economic loss when it can be injured by a pest that undergoes frequent and rapid race changes. Each change in race that causes existing cultivars to be susceptible necessitates the identification and utilization of new genes for resistance. The availability of new genes may be limited in some crop species. Stabilization of the racial structure of a pest population would be desirable.

Race stabilization could be achieved if prevalent races of a pest were maintained or new races could not develop. Both of these possibilities have been proposed as workable solutions to race change. They involve strategic use of major genes for resistance in the cultivars grown for commercial production.

Maintenance of Prevalent Races

The method by which cultivars with major gene resistance are utilized in commercial production may influence race stability. It has been proposed that race stabilization can be achieved by providing the prevalent races with sufficient susceptible plant material on which to survive. Two procedures have been implemented for providing susceptible plant material in a manner that would not result in major economic loss. One involves the alternate use of resistant and susceptible cultivars in a cropping sequence, and the other involves the use of mixtures of resistant and susceptible lines.

Alternation of Resistant and Susceptible Cultivars. A three-year cropping sequence is recommended in some areas of the southern United States to promote race stability of the soybean cyst nematode. A nonhost crop and a resistant soybean cultivar are grown in successive years to reduce the population of the pest to a level that will not cause major economic loss when a susceptible cultivar is planted. The susceptible cultivar is grown to permit the prevalent race to survive and maintain its dominant role in the population. There is still a lack of critical experimental evidence that substantiates the value of the cropping sequence for achieving race stability, even though the procedure is recommended to commercial growers.

Mixtures of Resistant and Susceptible Genotypes. Susceptible plants can be provided to a pest by planting a seed mixture of resistant and susceptible lines, rather than a pure cultivar that is homogeneous for resistance. The frequency of susceptible plants in the mixture is kept low enough to prevent an important economic loss. The value of seed mixtures in stabilizing races has not yet been determined.

Prevention of New Races

Race stabilization would be achieved if new races could not overcome the resistance in the plant. The pyramiding of major genes into a cultivar has been proposed as an effective way to prevent the expression of new virulent races (Nelson, 1973). The proposal is based on the concept that races of a pest can only be effective if they contain all the genes necessary for virulence. It has been suggested that the ability of a pest organism to survive in nature is reduced as the number of its virulence genes increases (Van der Plank, 1963). As a result, the probability is low that a new race would develop with virulence genes to overcome each major gene for resistance and still have the fitness needed for survival. The effectiveness of gene pyramiding to achieve race stability has not yet been proven.

MINIMIZING THE IMPACT OF NEW RACES

The impact of a new gene for virulence is greatest when a crop is uniformly susceptible. The diversity of major resistance genes in cultivars used for production can have a great influence on the vulnerability of a crop to important loss. Diversity among fields is achieved when cultivars with different major genes are grown. Diversity for major genes within a field is accomplished by planting a seed mixture made up of lines with different major genes for resistance.

Gene Deployment

The planned geographical distribution of major genes for use in cultivar development is referred to as gene deployment (Frey et al., 1973). Gene deployment has been proposed as an effective method of achieving diversity for major genes within and among geographical areas. It requires a number of genes with similar effectiveness for control of prevalent races, and cooperation among plant breeders and pathologists. Examples of gene deployment among all breeders of a crop within the United States are difficult to find. The primary reason is that major genes with equal resistance against prevalent races are difficult or impossible to find. When one gene is clearly superior to others, most breeders prefer to use it.

In the 1970s public soybean breeders in the northern United States agreed to use different plant introductions as sources of resistance to a new race of the pathogen that causes phytophthora rot. All of the plant introductions had similar resistance to the disease. Shortly after the program was implemented, another race was identified that attacked most of the plant introductions that had been distributed. As additional new races developed, the breeders were forced to utilize fewer sources of resistance, which reduced the possibility of effective gene deployment.

Multilines

The multiline concept was proposed as a means of providing protection within fields against serious injury from new races of diseases. A mixture of different major genes for resistance reduces the likelihood that a new race will be able to attack all the plants. The yield of plants that remain resistant would not be reduced by the new race. The resistant plants may reduce the damage to susceptible plants by reducing the spread of the disease organisms (Frey, 1982). Total crop loss also may be reduced by the ability of resistant plants to take advantage of the reduced competition from adjacent susceptible plants.

Resistant individuals in a mixture may reduce the speed with which a disease spreads. This possibility has been studied for wind-borne diseases of cereal grains, including crown rust of oat and leaf rust of wheat. These diseases are

spread by spores that land on a plant, infect it, and produce additional spores that are blown to other plants. Factors that influence the rapidity of disease spread through a group of plants were summarized by Van der Plank (1963) in the equation

$$x_t = x_0 e^{rt}$$

where x_t = total number of spores produced in a group of plants at a particular time, t
x_0 = number of spores that initially infected the group of plants
r = rate of increase in the number of new spores produced per day
e = constant = 2.718

Any reduction in x_0 or r can delay the spread of the pathogen within the field. A delay of several days during the critical period of seed filling can have an important impact on the productivity of susceptible plants.

The number of spores of rust that initially infect a group of plants, x_0, is influenced by the number of susceptible plants they can infect. A spore does not contribute to disease spread when it lands on a plant that it cannot infect. As the percentage of resistant plants in a field increases, the value of x_0 decreases.

The rate of increase in spore number per day, r, is influenced by the ability of a spore to infect a plant and produce new spores. It also is affected by the number of new spores that are able to infect plants. In a multiline, new spores that land on resistant plants are ineffective, which prevents them from contributing to any increase in the rate of spore production.

BREEDING FOR GENERAL RESISTANCE

General resistance is an important means of pest control in plants. It is considered more desirable than specific resistance because it provides some level of resistance to many races and is less vulnerable to genetic change in the pest. Breeding for general resistance is more difficult than for specific resistance because many genes with minor effects are involved.

Quantitatively, selection for general resistance is similar to breeding for yield and other agronomic characters. For example, effective improvement in general resistance has been accomplished by recurrent selection. Many alfalfa cultivars grown commercially are populations that were improved by recurrent phenotypic selection for disease and insect resistance.

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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.

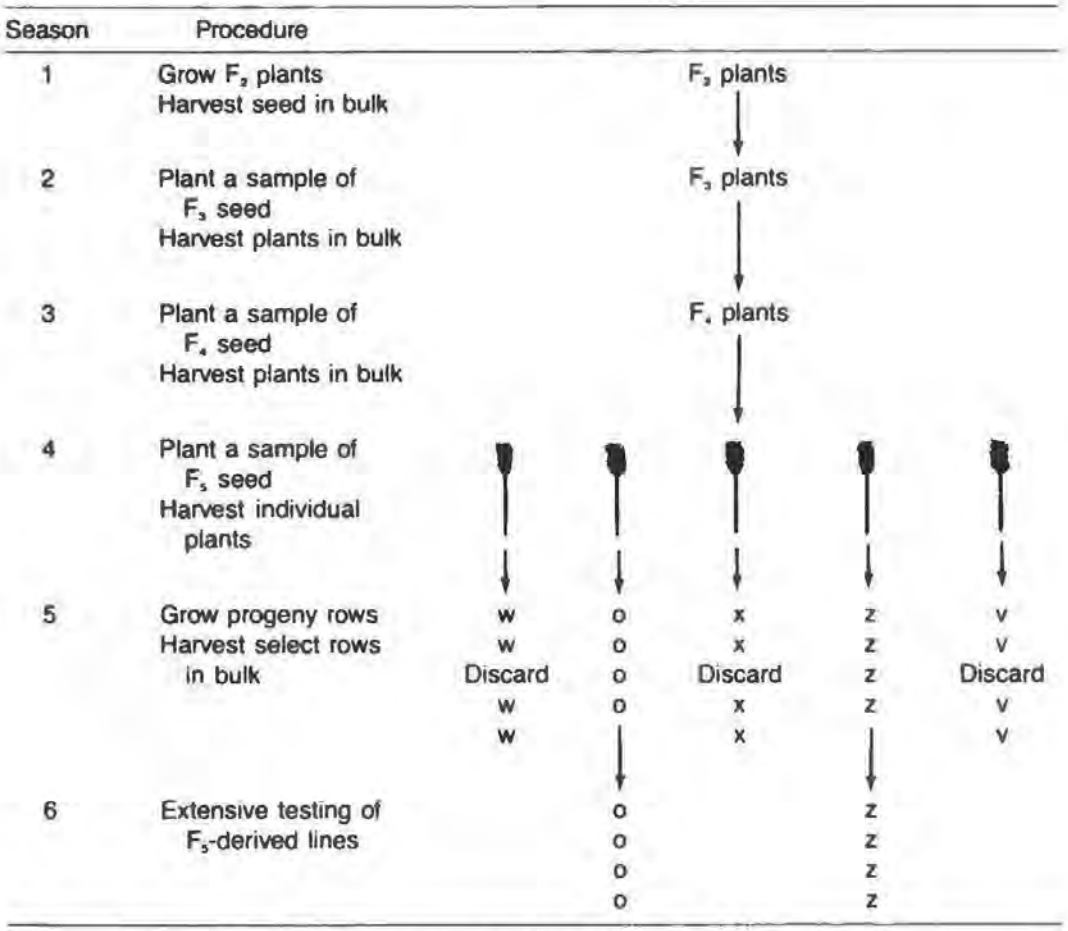


Figure 22-1 Illustration of the bulk method of inbreeding.

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.

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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.

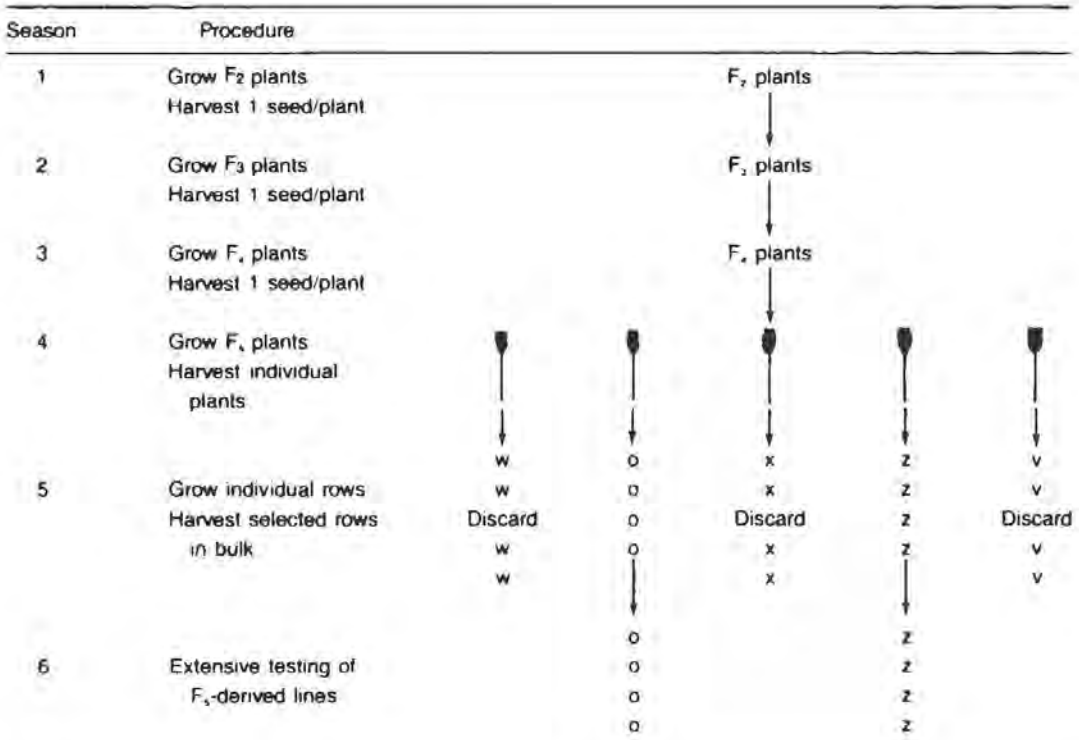


Figure 23-1 Illustration of the single-seed procedure for the single-seed descent method. (Courtesy of Fehr, 1978.)

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

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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.

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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

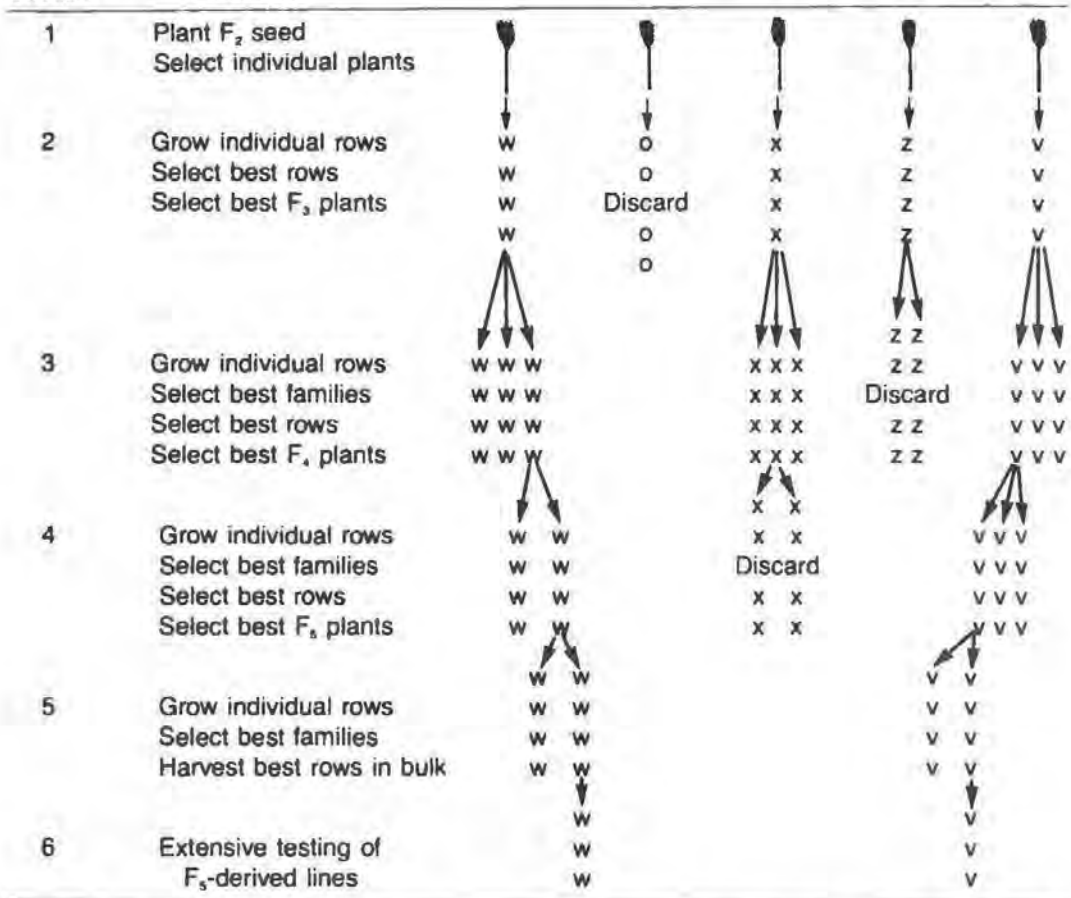


Figure 25-1 Illustration of the pedigree method. (Courtesy of Fehr, 1978.)

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

	Additive Genetic Variability	
Generation of Lines	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.

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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.

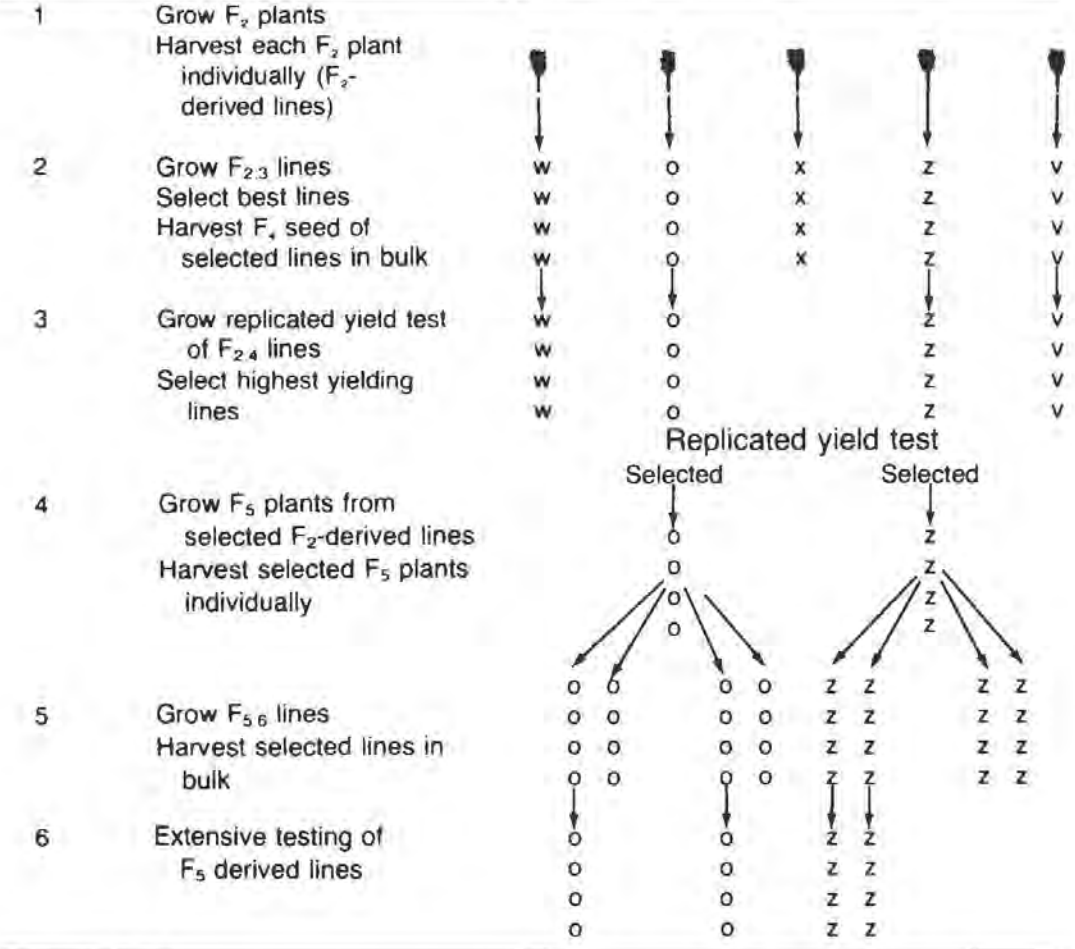
A breeder would not use the seed from the testing program to advance the population if seed mixtures or outcrossing was a problem or if a method of inbreeding other than the bulk method was preferred. Any method of inbreeding could be used in an independent program, including single-seed descent or pedigree.

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_1 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.

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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

Individual	Genotype		Phenotype
	Seedling	Endosperm	
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.

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CHAPTER TWENTY-EIGHT

Backcross Method

The backcross method is used to improve a characteristic of a cultivar for which it is deficient. The term backcrossing refers to the repeated crossing of hybrid progeny back to one of the parents. The parent contributing the genes that control the desired character is called the nonrecurrent or donor parent. Nonrecurrent indicates that the parent is used only once in the backcross procedure and it does not recur. The parent to which the genes are transferred is called the recurrent parent. Recurrent indicates that the parent is used (recurs) repeatedly in the backcrossing procedure.

The value of backcrossing for improvement of crop plants was suggested by Harlan and Pope (1922). They observed that backcrosses had been used for many years in animal breeding to fix desired characters. They indicate that the value of backcrosses for cultivar improvement of small grains had not been fully appreciated.

Harlan and Pope were concerned with the development of barley cultivars with smooth awns, instead of the rough awns that were present on existing cultivars. Imported barley cultivars had smooth awns, but they had objectionable characters, such as black seed. Crosses were made between the smooth-awned barleys and those of the commercially acceptable 'Manchuria' type, but none of the segregates was equal in yield to the 'Manchuria' parent. Harlan and Pope decided to transfer the smooth-awned character to 'Manchuria' by backcrossing. A white, six-rowed, smooth-awned segregate from a previous cross was used as the smooth-awned parent. After only one backcross, they noted that the progeny resembled 'Manchuria' more than observed previously from single-cross populations. The desirable progeny were obtained quickly and inexpensively with a small number of individuals. Subsequent studies in many crop species verified the value of backcrossing for developing improved crop cultivars.

IMPLEMENTATION

The recurrent parent in a breeding program should be a highly acceptable genotype, except for the trait that will be altered by backcrossing. The general principle is that the genotype obtained from backcrossing will not be improved for any character except the one being transferred from the donor parent. If the recurrent parent is inferior for traits in addition to the ones to be purposely altered by backcrossing, it will still be inferior for those traits when backcrossing is completed. Therefore, selection of the recurrent parent is a critical step in a backcrossing program.

The most desirable donor parent is one that has the alleles needed to improve the recurrent parent and that is not seriously deficient in other characters. The overall acceptability of the donor parent can influence the number of backcrosses required to recover the desirable characteristics of the recurrent parent.

The backcrossing procedure used will depend on the genetic control of the character that is to be transferred and the need for progeny testing of individuals to determine their genotype. Backcrossing is simplest when the desired character is controlled by a dominant allele and the character can be evaluated on a single-plant basis before flowering (Fig. 28-1). The following example involves four generations of backcrossing for a single gene controlling disease resistance.

Season 1: F_1 seed is obtained by crossing the susceptible recurrent parent (*rr*) to the resistant (*RR*) nonrecurrent parent. All F_1 seeds are *Rr*.

Season 2: F_1 plants (*Rr*) are crossed to the recurrent parent (*rr*) to obtain BC_1F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *rr*.

Season 3: The BC_1F_1 plants are evaluated before flowering and the susceptible plants (*rr*) are discarded. The resistant plants (*Rr*) are crossed to the recurrent parent to obtain BC_2F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *rr*.

Season 4: The BC_2F_1 plants are evaluated before flowering and susceptible plants (*rr*) are discarded. The resistant plants (*Rr*) are crossed to the recurrent parent to obtain BC_3F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *rr*.

Season 5: BC_4F_1 seeds are obtained in the same manner as described for seasons 3 and 4.

Season 6: The BC_4F_1 plants are evaluated before flowering and the susceptible plants (*rr*) are discarded. The resistant plants (*Rr*) are self-pollinated and the BC_4F_2 seeds are harvested at maturity.

Season 7: The BC_4F_2 plants on the average are 25 percent *RR*, 50 percent *Rr*, and 25 percent *rr*. The plants are evaluated before flowering, the susceptible plants (*rr*) are discarded, and the resistant plants (*RR* and *Rr*) are self-pollinated. The seed from each BC_4F_2 plant is harvested separately.

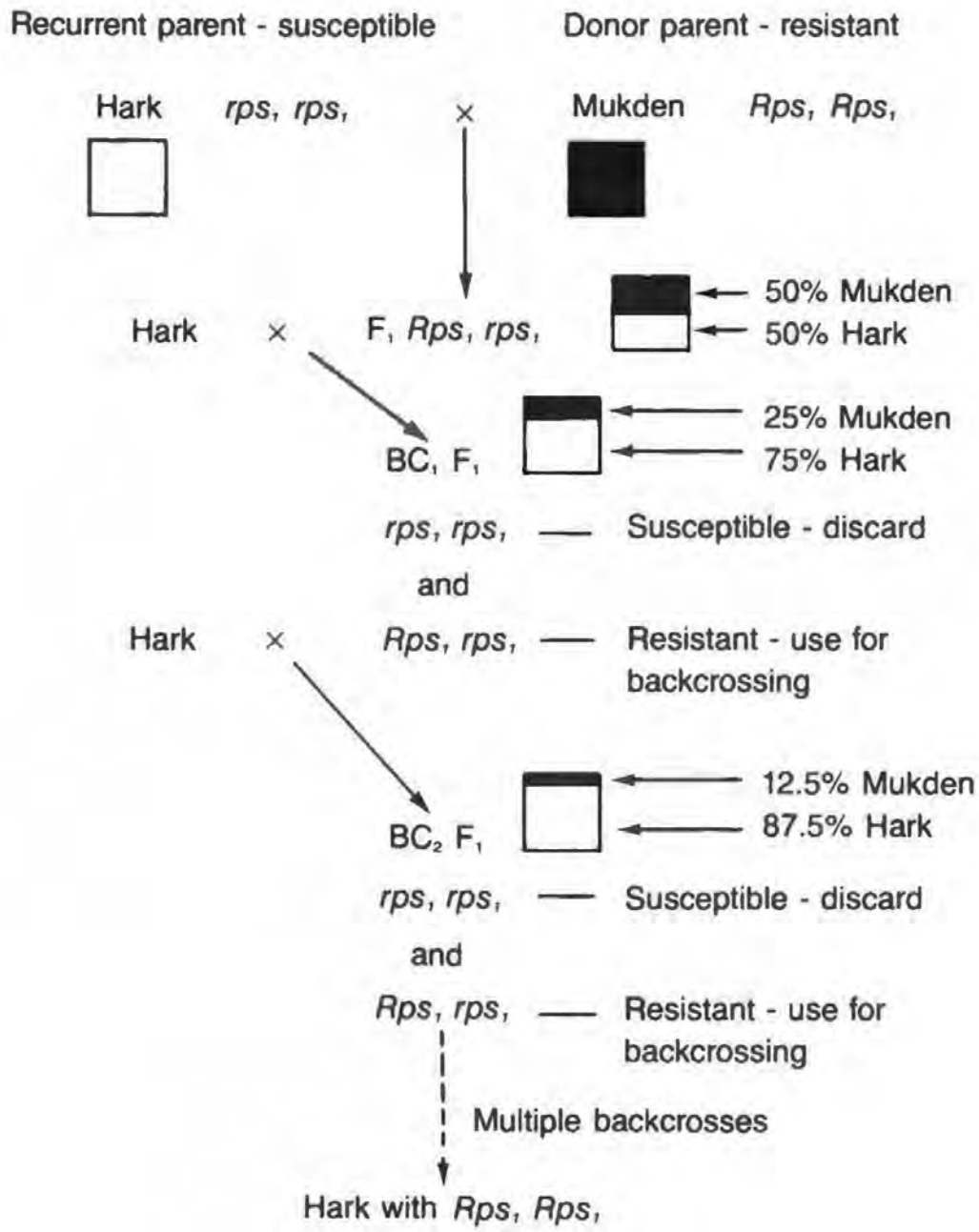


Figure 28-1 Schematic representation of transferring a gene from one cultivar to another by backcrossing. The donor parent 'Mukden' has resistance to race 1 of phytophthora rot (*Phytophthora megasperma* var. *sojae*) to which 'Hark' is susceptible. When backcrossing is completed, 'Hark' should have almost 100 percent of its original genes and also the gene for resistance to phytophthora rot. (Courtesy of Fehr, 1978.)

Season 8+: Each BC_4F_2 plant is progeny tested before flowering, and segregating progeny from *Rr* plants are discarded. Plants from BC_4F_2 individuals that are homozygous resistant *RR* are self-pollinated. During season 8 and subsequent seasons, the BC_4F_2 families are evaluated for important characters in comparison with the recurrent parent. The desirable ones are selected and bulked to replace the recurrent parent.

Some desired characters that are controlled by a dominant allele cannot be evaluated until after flowering is completed. In such cases, the procedure just described for a dominant character evaluated before flowering must be altered. One procedure that can be used is the following.

Season 1: F_1 seed is obtained by crossing the susceptible recurrent parent (*rr*) to the resistant nonrecurrent parent (*RR*). All F_1 seeds are *Rr*.

Season 2: F_1 plants are crossed to the recurrent parent (*rr*) to obtain BC_1F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *rr*.

Season 3: The BC_1F_1 plants are crossed to the recurrent parent. The character is evaluated before harvest, and susceptible plants and their BC_2F_1 seeds are discarded. The resistant plants (*Rr*) and their BC_2F_1 seeds (50 percent *Rr* and 50 percent *rr* on the average) are saved.

Seasons 4 and 5: The procedure described for season 3 is used to obtain BC_3F_1 and BC_4F_1 seeds.

Season 6: The BC_4F_1 plants are self-pollinated. The character is evaluated before harvest and susceptible plants are discarded. The BC_4F_2 seeds from resistant plants are harvested.

Season 7: The BC_4F_2 plants (25 percent *RR*, 50 percent *Rr*, and 25 percent *rr* on the average) are self-pollinated. The character is evaluated before harvest and susceptible plants are discarded. The seeds from each resistant BC_4F_2 plant (*RR* and *Rr*) are harvested separately.

Season 8+: Seeds from each resistant BC_4F_2 individual are planted and the plants in each row are self-pollinated. Rows segregating for the character are discarded. Selfed seeds from homogeneous resistant rows are harvested.

During season 8 and subsequent seasons, the resistant BC_4F_2 families are evaluated for important characters in comparison with the recurrent parent. The desirable ones are selected and bulked to replace the recurrent parent.

When the desired character is controlled by a recessive allele, a progeny test must be included in the backcrossing procedure. A progeny test also must be used when the character cannot be evaluated in the environment where the backcross is made, regardless if a dominant or recessive allele controls the desired phenotype. The following example describes the transfer of a recessive allele (*r*) in the shortest possible time when a progeny test is required to determine the genotype of a backcross progeny.

Season 1: F_1 seed is obtained by crossing the susceptible recurrent parent (*RR*) to the resistant nonrecurrent parent (*rr*). All of the F_1 seeds are *Rr*.

Season 2: F_1 plants are crossed to the recurrent parent (*RR*) to obtain BC_1F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *RR*.

Season 3: It is not possible to differentiate between the *Rr* and *RR* individuals because all have the dominant character. To complete the backcrossing in the fewest seasons, each BC_1F_1 plant is crossed to the recurrent parent to obtain BC_2F_1 seeds and is self-pollinated to obtain BC_1F_2 seeds for progeny testing.

Season 4: The BC_1F_2 seeds are used to progeny test each BC_1F_1 plant before the BC_2F_1 individuals begin flowering. BC_1F_1 plants that are homozgous susceptible (*RR*) will have all susceptible progeny. The BC_1F_2 progeny from heterozygous BC_1F_1 plants will segregate for resistant (*rr*) and susceptible plants. BC_2F_1 progeny from homozygous susceptible BC_1F_1 plants (*RR*) are all susceptible and are discarded. The BC_2F_1 progeny from heterozygous BC_1F_1 plants are crossed to the recurrent parent to obtain BC_3F_1 seeds and are self-pollinated to obtain BC_2F_2 seeds for progeny testing.

Season 5: The BC_2F_2 seeds are used to progeny test each BC_2F_1 plant before the BC_3F_1 individuals begin flowering. BC_3F_1 progeny from homozygous susceptible BC_2F_1 plants (*RR*) are all susceptible and are discarded. The BC_3F_1 progeny from heterozygous BC_2F_1 plants (*Rr*) are on the average 50 percent *Rr* and 50 percent *RR*. The BC_3F_1 plants are crossed to the recurrent parent to obtain BC_4F_1 seeds and are self-pollinated to obtain BC_3F_2 seeds for progeny testing.

Season 6: The BC_3F_2 seeds are used to progeny test each BC_3F_1 plant before their BC_4F_1 begin flowering. BC_4F_1 progeny from homozygous susceptible BC_3F_1 plants (*RR*) are all susceptible and are discarded. The BC_4F_1 plants obtained from crosses on heterozygous BC_3F_1 plants (*Rr*) are, on the average, 50 percent *Rr* and 50 percent *RR*, and all of them are self-pollinated. Each BC_4F_1 plant is harvested separately.

Season 7: Each BC_4F_1 plant is progeny tested. The progeny from homozygous susceptible BC_4F_1 plants (*RR*) are all susceptible and the family is discarded. The BC_4F_2 progeny from heterozygous BC_4F_1 plants (*Rr*) segregate for resistant (*rr*) and susceptible (*R*—) plants. If the progeny test can be completed before pollination, only the homozygous resistant (*rr*) plants are self-pollinated. If the character cannot be evaluated until after flowering, progeny from all BC_4F_1 plants are self-pollinated. The resistant BC_4F_1 plants are identified before harvest and the seed from each is kept separate.

Season 8+: During season 8 and subsequent seasons, the resistant BC_4F_2 families are evaluated for important characters in comparison with the recurrent parent. The desirable ones are selected and bulked to replace the recurrent parent.

The procedure as outlined must be modified slightly when used for situations in which the character cannot be evaluated in the same environment where the backcrosses are made. In seasons 4 to 6, the progeny test with BC_xF_2 seeds are conducted in the environment where the character can be evaluated, and the BC_xF_1 plants are grown in a separate environment where the backcrosses are made. The progeny test determines which BC_xF_1 plants should be used for crossing (seasons 4 and 5) or self-pollinated (season 6). In season 7, the BC_4F_2 progeny from heterozygous BC_4F_1 plants are self-pollinated and harvested separately. In season 8, each BC_4F_2 plant is progeny tested and only those that are homozygous for resistance (*RR* for a dominant allele or *rr* for a recessive allele) are saved. During season 8 and subsequent seasons, the progeny from homozygous resistant BC_4F_2 plants are evaluated for performance relative to the recurrent parent.

When a progeny test is required to determine the presence of the desired allele during backcrossing, the test can be conducted in one season and backcrossing in another. The procedure eliminates crosses on individuals that do not possess the allele, but increases the number of seasons required to complete the backcrosses. The following example is for transfer of resistance to disease controlled by a recessive allele. It is assumed that resistance cannot be evaluated until after pollination is completed and that four backcrosses will be used.

Season 1: F_1 seed is obtained by crossing the susceptible recurrent parent (*RR*) to the resistant nonrecurrent parent (*rr*). All of the F_1 seeds are *Rr*.

Season 2: F_1 plants are crossed to the recurrent parent (*RR*) to obtain BC_1F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *RR*.

Season 3: The BC_1F_1 plants are self-pollinated, and seed from each is harvested separately.

Season 4: BC_1F_2 progeny from each BC_1F_1 plant are grown in a row, plants are self-pollinated, resistant plants (*rr*) are identified within segregating rows, and the seed from resistant BC_1F_2 plants is harvested.

Season 5: Progeny from the homozygous resistant (*rr*) BC_1F_2 plants are crossed to the recurrent parent (*RR*) to obtain BC_2F_1 seeds (*Rr*).

Season 6: The BC_2F_1 plants are crossed to the recurrent parent to obtain BC_3F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *RR*.

Season 7: The BC_3F_1 plants are self-pollinated, and seed from each is harvested separately.

Season 8: BC_3F_2 progeny from each BC_3F_1 plant are grown in a row, plants are self-pollinated, resistant plants (*rr*) are identified within segregating rows, and the seed from resistant BC_3F_2 plants is harvested.

Season 9: Progeny from the homozygous resistant (*rr*) BC_3F_2 plants are crossed to the recurrent parent to obtain BC_4F_1 seeds (*Rr*).

Season 10: The BC_4F_1 plants are self-pollinated to obtain BC_4F_2 seed (25 percent *RR*, 50 percent *Rr*, and 25 percent *rr* on the average).

Season 11: BC_4F_2 plants are self-pollinated and the resistant ones are harvested individually.

Season 12+: During season 12 and subsequent seasons, the resistant BC_4F_2 families are evaluated for important characters in comparison with the recurrent parent. The desirable ones are selected and bulked to replace the recurrent parent.

GENETIC CONSIDERATIONS

Selection of the Female Parent

The cytoplasm of plant cells is obtained from the female gametes. Therefore, the cytoplasm of lines derived by backcrossing will be determined by the plants used as females in the initial cross and subsequent backcrosses. This is particularly important when cytoplasmic-genetic male sterility is used for hybrid seed production. Male-fertile inbred lines with normal cytoplasm and nonrestorer genes (B lines) are converted to sterile cytoplasm (A lines) so they can be used as the male-sterile female parent in a cross. In such a backcrossing program, a male-sterile line with sterile cytoplasm and nonrestorer genes is used as the female parent for the initial cross. The hybrid plants from the initial cross and all subsequent backcrosses will be male-sterile because they have the sterile cytoplasm.

If recovery of the cytoplasm of the nonrecurrent parent is desirable, even though cytoplasmic male sterility is not involved, the nonrecurrent parent must be used as the female parent in the initial cross. In all subsequent backcrosses, the recurrent parent must be used as the male.

The cytoplasm of the recurrent parent can be obtained in lines derived from backcrossing by using the recurrent parent as the female in the initial cross or in any of the backcross generations.

Probability of Transferring the Desired Genes

The probability of recovering desired genes during backcrossing depends on the expected frequency of individuals possessing the genes and the number of individuals available. It is possible to calculate the number of seeds or plants needed to have a given probability of success in recovering the desired genes.

Sedcole (1977) provided four methods for calculating the total number of plants needed to obtain one or more that have the desired genes for a given probability of success. The methods varied in the accuracy of the estimates and the complexity of the calculation. Using the most precise and complex method, he developed a table that can be used to determine the number of plants needed for most situations that would be encountered in backcrossing (Table 28-1).

Table 28-1 Total Number of Plants Needed to Obtain Required Number with Desired Genes

p^*	$q^\dagger$	r (Number of Plants to Be Recovered)								
		1	2	3	4	5	6	8	10	15
0.95	1/4	3	5	6	8	9	11	14	17	25
	1/5	5	8	11	13	16	18	23	28	40
	1/8	8	13	17	21	25	29	37	44	62
	1/14	11	18	23	29	34	40	50	60	84
	1/28	23	37	49	60	71	82	103	123	172
	1/56	47	75	99	122	144	166	208	248	347
	1/112	95	150	200	246	291	334	418	500	697
	1/64	191	302	401	494	584	671	839	1002	1397
0.99	1/4	4	6	8	9	11	13	16	19	27
	1/5	7	11	14	17	19	22	27	33	45
	1/8	12	17	22	27	31	35	44	52	71
	1/14	17	24	31	37	43	49	60	70	96
	1/28	35	51	64	77	89	101	124	146	198
	1/56	72	104	132	158	182	206	252	296	402
	1/112	146	210	266	318	368	416	508	597	809
	1/64	293	423	535	640	739	835	1020	1198	1623

*p = probability of recovering r plants with the desired genes.

†q = frequency of plants with desired genes.

Source: Sedcole, 1977.

For situations not covered by Table 28-1, Sedcole (1977) indicated that a reasonably reliable method of calculating the number of plants needed can be obtained from the equation

$$n = \frac{[2(r - 0.5) + z^2(1 - q)] + z[z^2(1 - q)^2 + 4(1 - q)(r - 0.5)]^{1/2}}{2q}$$

where n = total number of plants necessary
r = required number of plants with desired genes
q = frequency of plants with desired genes
p = probability of recovering required number of plants with desired genes
z = value that is function of probability (p).

The value of z is 1.645 for $p = 0.95$ and 2.326 for $p = 0.99$. Sedcole provided the following example, which assumes that $r = 15$, $q = 1/64$, and $p = 0.95$, ($z = 1.645$).

$$n = \frac{\left[[2(14.5) + 1.645^2\left(\frac{63}{64}\right)\right] + 1.645\left[1.645^2\left(\frac{63}{64}\right)^2 + 4\left(\frac{63}{64}\right)(14.5)\right]^{1/2}}{2(1/64)}$$

$$n = 1420$$

Germination percentage must be considered when determining the number of seeds needed to provide a certain number of plants. The equation is

$$\text{Number of seeds} = \frac{\text{number of plants needed}}{\text{germination percentage}}$$

For example, if 100 plants are required and the germination percentage is 80 percent, the calculation is

$$\text{Number of seeds} = \frac{100}{0.80} = 125$$

Calculations involved in backcrossing will be illustrated here by making the calculations associated with the transfer of a desired character (disease resistance) that is controlled by a single recessive allele.

Season 1: Crosses are made between the nonrecurrent (*rr*) and the recurrent (*RR*) parents. The number of F_1 seeds that should be obtained will depend on the number of hybrid plants required in season 2 for crossing to obtain the required number of BC_1F_1 seeds. We will assume that the BC_1F_1 seeds can be obtained on one F_1 plant and that the germination percentage for F_1 seeds is 80 percent. The minimum number of F_1 seeds that should be obtained is

$$\frac{1\ F_1\ \text{plant}}{0.80} = 2\ F_1\ \text{seeds}$$

Therefore, at least two F_1 seeds should be obtained in season 1.

Season 2: F_1 plants (*Rr*) are crossed to the recurrent parent (*RR*) to obtain BC_1F_1 seeds, of which an average of 50 percent are *Rr* and 50 percent are *RR*. How many BC_1F_1 seeds should be obtained? The answer is a function of (a) the number of heterozygous BC_1F_1 plants that are desired for crossing in season 3, (b) the probability of success required for obtaining the desired number of heterozygous BC_1F_1 plants, and (c) the germination percentage. We will assume that three heterozygous BC_1F_1 plants are desired and that the probability of success is 99 percent. When $p = 0.99$, $r = 3$, and q = frequency of *Rr* individuals = 1/2, the number of BC_1F_1 plants that should be available in season 3 is 14 (Table 28-1). With a germination percentage of 80 percent, the number of BC_1F_1 seeds required is 14/0.80 = 18 seeds.

Season 3: Each BC_1F_1 plant (*Rr* or *RR*) is crossed to the recurrent parent to obtain BC_2F_1 seeds. How many BC_2F_1 seeds should be obtained on each plant? The answer is a function of (a) the number of heterozygous BC_2F_1 plants that are desired for crossing in season 4, (b) the probability of success required, and (c) the germination percentage. We will assume that two heterozygous BC_2F_1 plants are desired from each heterozygous BC_1F_1 plant and that the probability of success is 95 percent. If a heterozygous BC_1F_1 plant (*Rr*) is crossed to the recurrent parent, the fre-

quency of heterozygous (*Rr*) BC_2F_1 seeds is 1/2. When $p = 0.95$, $r = 2$, and $q = 1/2$, the number of BC_2F_1 plants required is eight. With a germination percentage of 80 percent, the number of BC_2F_1 seeds required from each BC_1F_1 plant is 8/0.80 = 10 seeds.

Season 4: BC_1F_2 seeds are used to progeny test each BC_1F_1 plant. How many BC_1F_2 plants should be grown to evaluate adequately the genotype of each BC_1F_1 individual? Each heterozygous *Rr* plant will have progeny that segregate, on the average, 3/4 susceptible (*RR* or *Rr*) and 1/4 resistant (*rr*). The number of BC_1F_2 plants that should be grown will depend on the minimum number of resistant plants that must be found in the progeny and the probability of success. When $p = 99$, $r = 2$, and $q = 1/4$, the number of plants is 24. With a germination percentage of 80 percent, the number of BC_1F_2 seeds that should be planted for the progeny test is 24/0.80 = 30.

The illustrations presented thus far have used characters controlled by a single gene. If two or more genes control a character, the number of plants required each backcross generation to transfer the desired genes successfully increases substantially. For example, assume that the desired character is controlled by dominant alleles at two loci. The initial cross between the nonrecurrent parent *AABB* and the recurrent parent *aabb* provides heterozygous F_1 plants *AaBb*. When the F_1 plants are crossed to the recurrent parent, how many BC_1F_1 plants must be obtained to be 99 percent sure that three plants have both dominant alleles? The frequency of *AB* gametes from the F_1 plants is 1/4, which is the frequency of *AaBb* plants expected in the BC_1F_1. When $p = 0.99$, $q = 1/4$, and $r = 3$, the number of BC_1F_1 plants required is 31 (Table 28-1). In contrast, obtaining the same number of heterozygous BC_1F_1 individuals for a single gene would require only 14 plants.

Recovery of Genes from the Recurrent Parent

The objective of backcrossing is to recover the genes of the recurrent parent, except for the ones being transferred from the donor parent. The average rate of recovery depends on the amount of selection for characteristics of the recurrent parent that is practiced during backcrossing, and the effect of linkage.

In the absence of selection and linkage, the average percentage of genes from the recurrent parent increases each backcross by one-half of the percentage of germplasm of the nonrecurrent parent that was present in the previous backcross generation (Table 28-2). The general equation for average recovery of the recurrent parent is $1 - (1/2)^{n+1}$, where n is the number of backcrosses to the recurrent parent. For the initial cross, $n = 0$; for the first backcross, $n = 1$; and so forth.

The term average recovery is used because within each backcross generation there is a range among plants for the number of genes from the recurrent parent that they possess. This variation among plants is reflected by the frequency of

Table 28-2 Average Recovery of Genes from Recurrent Parent During Backcrossing

	Percentage of Parentage	
Generation	Recurrent	Nonrecurrent
F_1	50	50
BC_1F_1	75	25
BC_2F_1	87.5	12.5
BC_3F_1	93.75	6.25
BC_4F_1	96.875	3.125
BC_5F_1	98.4375	1.5625

individuals in each generation that are homozygous for the genes of the recurrent parent. The general equation used (Allard, 1960) is

$$\text{Proportion of homozygous individuals} = \left(\frac{2^m - 1}{2^m}\right)^n$$

where m is the number of backcrosses to the recurrent parent and n is the number of genes for which the recurrent and nonrecurrent parents had different alleles (number of heterozygous loci). For the initial cross, $m = 0$; for the first backcross, $m = 1$; and so forth.

If the nonrecurrent and recurrent parents have different alleles at 10 loci, only 0.1 percent of the BC_1F_1 plants, 6 percent of the BC_2F_1, and 26 percent of the BC_3F_1 will be homozygous for the 10 alleles of the recurrent parent (Table 28-3). The remainder of the plants will have varying frequencies of the 10 alleles; consequently, there can be considerable variation in the phenotype of plants, especially in the early generations. Selection of plants for backcrossing that resemble the recurrent parent can increase the rate of recovery of its genes. For that reason, it is useful to obtain more than the minimum number of plants needed to be certain the desired genes are present. For example, if the frequency of plants with the desired genes is $q = 1/2$ and the probability of success is $p = 0.95$, a minimum of five plants is needed to obtain the desired genes. If 40 backcross individuals are obtained, approximately 15 should have the desired genes. Se-

Table 28-3 Percentage of Individuals Homozygous for Alleles of Recurrent Parent in Different Backcross Generations

Number of Genes with Different Alleles	Backcross Generation					
	1	2	3	4	5	6
	%					
1	50	75	88	94	97	98
2	25	56	77	88	94	97
5	3	24	51	72	85	92
10	0.1	6	26	52	73	85

lection among the 15 plants for those most like the recurrent parent can hasten the recovery of genes from the recurrent parent.

Effect of Linkage on Recovery of Genes of the Recurrent Parent

The impact of linkage on recovery of genes of the recurrent parent was discussed in the original paper on backcrossing by Harlan and Pope (1922). They noted that backcrossing provides a good opportunity for crossing over to occur between the desired genes from the nonrecurrent parent and undesirable ones linked to them. Assume that resistance to disease (R) is linked to an undesirable gene (d) in the nonrecurrent parent and that the recurrent parent has the alleles rD. For recovering the desired RD gamete, a crossover between Rd is effective in the heterozygous genotype $\frac{Rd}{rD}$, but not in the homozygous individuals $\frac{Rd}{Rd}$ or $\frac{rD}{rD}$. In each backcross generation, Rd gametes from the backcross progeny are united with rD gametes from the recurrent parent to produce $\frac{Rd}{rD}$ individuals. In contrast, selfing in a population reduces the percentage of heterozygous individuals, thereby reducing the possibility of effective crossing over.

Allard (1960) indicated that the probability of eliminating an undesirable gene linked to the desired one could be expressed by the equation

$$1 - (1 - p)^{m+1}$$

where p is the recombination fraction between the linked genes and m is the number of backcrosses. For example, if the recombination fraction were 0.10, the probability of eliminating the undesirable gene without selection during five backcrosses would be

$$1 - (1 - 0.10)^{5+1} = 0.47$$

Table 28-4 Effect of Linkage on Probability of Eliminating Undesirable Gene (b) Linked to Desirable Gene (A)*

Recombination Fraction	Probability that Undesirable Gene Will Be Eliminated	
	With 5 Backcrosses	With Selfing
0.50	0.98	0.50
0.20	0.74	0.20
0.10	0.47	0.10
0.02	0.11	0.02
0.01	0.06	0.01
0.001	0.006	0.001

*It is assumed that selection is practiced for A but not for b.

Source: Allard, 1960.

The probability of eliminating an undesirable gene *b* linked to a desirable gene *A* by selfing and backcrossing when no selection is practiced is provided in Table 28-4.

Retaining the Heterogeneity of the Recurrent Parent

Inbred lines of self- and cross-pollinated crops generally have some genetic heterogeneity, even though it may not be readily visible. If it is considered important to retain the heterogeneity in the backcross-derived cultivar, a large number of plants from the recurrent parent should be used during the last backcross. The number that should be used is not fixed; however, 30 generally is considered a minimum.

Backcrossing for Quantitative Characters

The ease and success of backcrossing is influenced by (a) the number of genes controlling a character and (b) the role of the environment on expression of the genes. As the number of genes increases, the proportion of the desired genotypes decreases. As the proportion of a desired genotype decreases, the number of plants that must be grown to recover that genotype increases. This can be understood by examining Table 28-1 for different proportions of q. Therefore, one problem associated with backcrossing for quantitative characters is the large population size that must be used.

A second difficulty with backcrossing for quantitative characters is the influence of environment on expression of the desired genes. Success in backcrossing is dependent on the ability to identify the desirable genotypes in each generation. When the environment has a large influence on expression of the character, such identification can be difficult.

Success in the transfer of quantitative characters can be influenced by (a) selection of the recurrent parent, (b) self-pollination, and (c) progeny testing.

1. *Selection of the nonrecurrent parent:* It can be difficult, if not impossible, to completely transfer the desired character as found in the nonrecurrent parent. For that reason, it is helpful whenever possible to choose a nonrecurrent parent that is more extreme for a character than is desired in the backcross-derived lines. For example, assume that the objective is to make a cultivar 10 days earlier in maturity. If a nonrecurrent parent is chosen that reaches maturity only 10 days earlier than the recurrent parent, it may be possible to obtain lines that are 7 or 8 days earlier than the recurrent parent, but extremely difficult to achieve any that are 10 days earlier in maturity. The alternative is to use a nonrecurrent parent that is 20 days earlier in maturity. A breeder can lose half the earliness of the nonrecurrent parent and still reach the desired objective.

As for a qualitative character, the nonrecurrent parent should resemble the recurrent parent as much as possible for all characters except the one being transferred. This may reduce the number of backcross generations required to recover lines with the desirable characteristics of the recurrent parent.

2. *Self-pollination:* The genes controlling the desired character are heterozygous after each backcross, and BCF_1 plants differ by the number of the desired alleles from the nonrecurrent parent that they possess. Because the alleles from the recurrent parent are common to all plants, the variability among BCF_1 plants for the character of interest may be limited. Self-pollination of plants after each backcross increases the number of different genotypes by the formation of individuals homozygous for alleles. Selfed individuals with a high frequency of desired alleles, some in the homozygous condition, should be more readily detected than BCF_1 individuals that have only heterozygous loci. Each generation of self-pollination increases the formation of homozygous loci and the variability among individuals; therefore, selection may be practiced among F_2 plants or those in later generations.
3. *Progeny testing:* Selection on a single-plant basis can be relatively ineffective for characters that are strongly influenced by the environment. A replicated progeny test of selfed individuals can improve the reliability of information about the genetic potential of a single plant and increase the chance of using genotypes for backcrossing that have the highest frequency of the desired alleles. If plants are self-pollinated either manually or naturally during the progeny test, single-plant selection within superior lines can further enhance the selection of superior individuals for backcrossing.

General considerations in transferring a quantitative character are outlined here for an inbred line or cultivar. The goal of the backcrossing program in this example is to improve the protein content of the seed of an inbred line from the present level of 20 percent to the desired level of 25 percent. A nonrecurrent parent is chosen that has 30 percent protein and that resembles the recurrent parent as much as possible for other characteristics.

Season 1: F_1 seeds are obtained by crossing the nonrecurrent (30 percent protein) and recurrent (20 percent protein) parents.

Season 2: The F_1 plants are self-pollinated to obtain F_2 seeds.

Season 3: Plants of the F_2 generation and the recurrent and nonrecurrent parents are grown and harvested separately. Seed of each F_2 plant is evaluated for protein content, and its content is compared with the content of the recurrent and nonrecurrent parents. F_2 plants with the highest seed protein are retained for progeny testing in season 4 as $F_{2:3}$ lines.

Season 4: A replicated test is grown for the $F_{2:3}$ lines that were saved in season 3. The $F_{2:3}$ lines with the highest seed protein content are identified for use as parents in the first backcross during season 5.

Season 5: The F_2-derived lines with high seed protein content are crossed to the recurrent parent to obtain BC_1F_1 seeds.

Season 6: The BC_1F_1 plants are self-pollinated to obtain BC_1F_2 seeds.

Season 7: Plants of the BC_1F_2 generation and the parents are grown and harvested separately. BC_1F_2 plants with the highest protein are identified for progeny testing in season 8 as $BC_1F_{2:3}$ lines.

Season 8: A replicated test is grown for the $BC_1F_{2:3}$ lines that were saved in season 7. The $BC_1F_{2:3}$ lines with the highest protein content are identified for use as parents in the second backcross during season 9.

Seasons 9–12: The second backcross is made and the progeny evaluated with the procedure used in seasons 5 to 8. All subsequent backcrosses are made and evaluated in the same manner.

After the desired number of backcrosses has been made, the progeny with high protein and the characteristics of the recurrent parent are tested for yield and other important characters.

Lines with the desired characteristics can be bulked and used as an improved version of the recurrent parent.

The procedure adopted for backcrossing a quantitative character will vary with the heritability of the character involved and the importance of time in completing the work.

1. If satisfactory evaluation of the character being transferred can be made on a single-plant basis, the season of replicated progeny testing can be eliminated. If single-plant evaluation is of limited value, replicated progeny tests will be the only method of identifying lines with the desired characteristics.
2. Selection on a single-plant basis within the progeny of F_2 plants may be helpful in identifying segregates that have a superior level of the character.
3. If time is an important consideration, backcrosses can be made on plants or lines during the season of their evaluation. Backcross seed would only be saved from those plants or lines that had the desired characteristic.

Number of Backcrosses

The number of backcrosses will depend on (a) the importance of recovering characteristics of the recurrent parent, (b) the amount of selection imposed during backcrossing, and (c) the similarity of the nonrecurrent parent to the recurrent one and (d) the extent of linkage between desirable and undesirable genes. There may be as few as one backcross or more than five.

There usually are at least four backcrosses when it is important that the recovered lines be essentially identical to the recurrent parent except for the characteristic being transferred. This is particularly true when the cultivar derived from backcrossing is given the same name as the recurrent parent, to which a number or letter has been added.

A relatively large number of backcrosses is required when the nonrecurrent parent is markedly inferior to the recurrent parent in one or more characters. Use of plant introductions or wild relatives of the cultivated species as nonrecurrent parents can introduce undesirable characters that require multiple backcrossing before their elimination. Selection can aid in rapid recovery of characteristics of the recurrent parents.

Only one or a few backcrosses are used when the new cultivar does not have to resemble the recurrent parent in all characteristics. Allard (1960) suggested that development of cultivars after only a few backcrosses should be called the backcross pedigree method, instead of backcrossing. He suggested that the term backcrossing be used only when the objective is to recover all of the features of the recurrent parent. The advantage of limited backcrossing is that the possibility exists for identifying segregates that are superior to the recurrent parent for characters other than the one being transferred. The potential for transgressive segregation decreases with each generation of backcrossing.

In transferring quantitative characters, it can be difficult to retain a high level of the desired character through multiple backcrosses. One possibility for reducing the number of backcrosses is to select in each backcross generation for the character being transferred, then evaluate the selected individuals in replicated tests for other important characters. Backcrossing can be discontinued whenever an individual with the desired characteristics is recovered, regardless of the number of backcrosses involved.

Transferring Multiple Characters

It can be desirable to improve a cultivar simultaneously for more than one character. An example is the development of a cultivar that has different genes for disease resistance, sometimes referred to as pyramiding. There are two options for transferring multiple characters: (a) transfer the genes simultaneously in one backcrossing program or (b) transfer the genes in independent backcrossing programs and combine them into one individual at the end.

The problem with transferring several genes simultaneously is the large number of backcross seed that must be obtained to be certain that a genotype with all the desired genes is present. To illustrate, assume that four independent genes for disease resistance, *FHJN*, are to be transferred simultaneously. If a heterozygous individual, *FfHhJjNn*, were backcrossed to the recurrent parent (*ffhhjjnn*), the frequency of an *FfHhJjNn* individual would be $(1/2)^4 = 1/16$. For a 99 percent probability that at least one heterozygous individual would be recovered, 72 backcross progeny would be required (Table 28-1). In contrast, if each gene was transferred independently, the frequency of recovering a heterozygous individual for one gene would be 1/2. Seven backcross progeny would be required to be 99 percent certain of recovering the heterozygous individual, for a total of $7 \times 4 = 28$ backcross progeny for the four independent backcross programs.

Another potential problem with the simultaneous transfer of multiple char-

acters is the difficulty of evaluating one individual for more than one character at a time. When there are independent backcrossing programs for each character, individuals are only evaluated for one character at a time.

When characters are transferred in independent programs, the procedure for each character is one of those that have already been discussed. After the backcross-derived lines from the independent programs are available, their genes must be combined into a single individual. An example of a procedure to combine four dominant alleles in one individual follows.

Season 1: The following crosses are made: (a) *FFhhggnn* × *ffHHggnn* to obtain *FfHhggnn* individuals and (b) *ffhhGGnn* × *ffhhggNN* to obtain *ffhhGgNn* individuals.

Season 2: The F_1 plants from the crosses in season 1 are crossed, *FfHhggnn* × *ffhhGgNn*. The frequency of *FfHhGgNn* individuals would be $(1/2)^4 = 1/16$; therefore, 72 hybrid seeds would be required for 99 percent probability that at least one individual had the desired genotype.

Season 3: Each F_1 plant obtained in season 2 is evaluated for resistance to the four races, and selected *FfHhGgNn* individuals are self-pollinated.

Season 4: F_2 progeny from self-pollinated *FfHhGgNn* plants are evaluated and plants resistant to the four races are self-pollinated and harvested separately. The frequency of *F_ H_ G_ N_* plants among the F_2 progeny is $(3/4)^4 = 81/256 = 32\%$, and the frequency of *FFHHGGNN* plants is $(1/4)^4 = 1/256 = 0.4\%$.

Season 5: F_3 progeny from selected F_2 plants are grown. Plants within each row are evaluated, and resistant plants are self-pollinated and harvested separately. The frequency of *FFHHGGNN* F_3 plants has increased through self-pollination.

Season 6: F_4 progeny from selected F_3 plants are grown. Each line is evaluated to identify those homozygous for the four dominant alleles. Such lines are self-pollinated and harvested separately.

Season 7: The $F_{3:5}$ lines are evaluated for important agronomic characters, and self-pollinated seeds of the acceptable ones are bulked to form the backcross-derived line that will replace the recurrent parent.

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CHAPTER TWENTY-NINE

Types of Cultivars

The types of cultivars used to propagate a species for commercial production have a major impact on the methods utilized by the plant breeder for genetic improvement. For example, tests of combining ability are a major consideration in the development of inbred lines used in the production of hybrid cultivars, but are not of importance in the development of a self-pollinated cultivar. Methods of inbreeding are important for the development of inbred lines or self-pollinated cultivars, but not for the selection of a clone to be used as a vegetatively propagated cultivar.

Classification of the different types of cultivars provides an overview of the alternatives available for commercial propagation of crop species. The variability among species for modes of reproduction makes it difficult to develop absolute definitions for each cultivar class. Nevertheless, a committee of the U.S. Department of Agriculture has developed useful guidelines for classifying cultivated plant populations. The committee report and the International Code of Nomenclature for Cultivated Plants are the basis of the information outlined in this chapter on types of plant cultivars.

The terms variety and cultivar (cultivated variety) will be considered equivalent. A cultivar is a group of plants with characteristics that are distinct, uniform, and stable. Distinct indicates that the cultivar can be differentiated by one or more identifiable morphological, physiological, or other characteristics from all other known cultivars. Uniform designates that variation among the plants of a cultivar for distinctive characteristics can be described. Stable indicates that the

cultivar will remain unchanged to a reasonable degree of reliability in its distinctive characteristics and its uniformity when reproduced or reconstituted.

CLONAL CULTIVARS

A clonal cultivar consists of one clone or several closely similar clones that are propagated by asexual means. The methods of propagation include cuttings, tubers, bulbs, rhizomes, and grafts. A clonal cultivar could be propagated asexually from seed produced by obligate apomixis. Examples of clonal cultivars include 'Meyer' zoysiagrass, 'Elberta' peach, 'Russett Burbank' potato, 'Coastal' bermudagrass, 'Peace' rose, 'Iceberg' chrysanthemum, and 'Higgins' buffelgrass.

LINE CULTIVARS

Line cultivars consist of a group of plants of self- or cross-pollinated species that have largely the same genetic background, defined as a theoretical coefficient of parentage of 0.87 or higher (Kempthrone, 1957). The cultivars are maintained by self-pollination or sib mating. Examples of line cultivars from normally self-pollinated crops include 'Gaines' wheat, 'Tendercrop' snap bean, and 'Wayne' soybean. Examples of line (inbred) cultivars from normally cross-pollinated crops include 'MSU-713-5' gynoecious cucumber, 'WF9' maize, 'Nittany Lion Red' geranium, and 'B2215C' onion. Classification of single-line facultative apomicts is complicated by variation among cultivars in the percentage of seed produced asexually. Cultivars with 95 percent apomixis for single-line facultative apomicts are considered pure lines. In cases where it is not possible to achieve 95 percent apomixis, single-line facultative apomicts with a level of apomixis as low as 80 percent may be classed as line cultivars even though the plants may differ in morphologial characteristics. Examples of single-line facultative apomicts meeting the 95 percent apomixis requirement include 'Pennstar' and 'Merion' Kentucky bluegrass. Cultivars with below 95 percent apomixis include 'Adelphi' and 'Bristol' Kentucky bluegrass.

OPEN-POLLINATED CULTIVARS OF CROSS-POLLINATED CROPS

Open-pollinated cultivars consist of normally cross-pollinated plants selected to a standard that allows variation but in which the cultivars have one or more characteristics that differentiate them from other cultivars. Examples include 'Kenland' red clover, 'Nordan' crested wheatgrass, 'Yellow Bermuda' onion, 'Elbon' rye, 'Thumbelina' zinnia, 'Poinsett' cucumber, and 'Golden Bantam' sweet corn.

SYNTHETIC CULTIVARS

First-Generation Synthetic Cultivars (Syn 1)

A Syn 1 cultivar consists of first-generation progenies derived by intercrossing a specific set of clones or seed-propagated lines. A Syn 1 cultivar may be used for normally cross-pollinated crops or for self-pollinated crops into which mechanisms have been introduced to maximize cross-pollination, such as male sterility or self-incompatibility. The cultivars usually contain mixtures of seed resulting from cross-, self-, and sib-pollination. Syn 1 cultivars consist only of first-generation progenies after intercrossing and cannot be reproduced from seed of the first generation. Examples include 'Gahi' pearl millet, 'Vitagraze' rye, and 'Tempo' alfalfa.

Advanced Generation Synthetic Cultivars (Beyond Syn 1)

Synthetic cultivars may consist of generations of open-pollinations advanced beyond the Syn 1 generation. The Syn 2 generation is produced from the Syn 1 generation, the Syn 3 is produced from the Syn 2, and so forth. The cultivars usually are stable for only a limited number of generations of reproduction, and are reconstituted from the original parental lines or clones. Examples include 'Ranger' and 'Moapa' alfalfa, 'Saratoga' bromegrass, and 'Pennlate' orchardgrass.

HYBRID CULTIVARS (F_1)

Hybrid cultivars consist of first-generation (F_1) progenies from a cross produced through controlling the pollination between (a) two inbred lines (single cross), (b) two single crosses (double cross), (c) a single cross and an inbred line (three-way cross), (d) an inbred line or a single cross and an open-pollinated or a synthetic cultivar, or (e) two selected clones, seed lines, cultivars, or species. The hybrid cultivar cannot be reproduced from seed of the hybrid generation. Examples include 'Hybrid-7' spinach, 'US 13' hybrid maize, 'RS-610' hybrid grain sorghum, 'Moreton' hybrid cabbage, 'Valley' hybrid sunflower, and 'Picadilly' hybrid cucumber.

F_2 CULTIVARS

An F_2 cultivar is derived by self-pollination or open-pollination of an F_1 hybrid. The cultivar cannot be perpetuated by growing additional generations. Examples

include 'Foremost F_2' tomato, 'Market Pride' cantaloupe, 'Violet Blue' petunia, and 'Seven-Eleven' pansy.

COMPOSITE-CROSS POPULATIONS

A composite-cross population is generated by hybridizing more than two cultivars or lines of normally self-pollinated plants and propagating successive generations of the segregating population in bulk in specific environments so that natural selection is the principal force acting to produce genetic change. Artificial selection also may be imposed on the population. The resulting population is expected to have a continuously changing genetic makeup. Breeder seed is not maintained as originally released. Examples include 'Harland' barley and 'Mezcla' lima bean.

MULTILINES

A multiline or blend is a seed mixture of cultivars or lines each present in excess of 5 percent of the whole. A multiline may consist of two or more near-isogenic lines of normally self-pollinated plants that are similar in most characteristics but differ in a limited number of distinctive characteristics, such as disease resistance. The term multiline also has been used to describe a seed mixture of two or more unrelated lines. A multiline is derived by growing the component lines separately and then compositing the seed in a predetermined percentage of each component. If the components differ in seed productivity in a mixed population, the genetic composition of the multiline will change each generation of reproduction. Examples include 'Webster' blend oat and 'Miramar-63' wheat.

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CHAPTER THIRTY

Development of Asexually Propagated Cultivars

The cultivars of some important crop species are planted commercially from vegetative propagules or seed derived by apomixis. Asexually propagated cultivars may be obtained by introduction from another country, by hybridization between genotypes, or by mutation of a genotype. The primary focus in this chapter will be on cultivar development by hybridization. Population formation and selection by mutation breeding are discussed in Chap. 20.

The three steps in development of an asexually propagated cultivar are (a) development of a source of genetic variability, (b) evaluation of individuals (clones) from the population, and (c) multiplication of vegetative propagules or apomictic seed of a new cultivar for commercial use.

SOURCES OF GENETIC VARIABILITY

In species that are propagated asexually, there is a positive relationship between the productivity of an individual and its level of heterozygosity. Because of this relationship, the emphasis in developing sources of genetic variability is to maximize the heterozygosity of individuals in the population. Asexual propagation ensures that the heterozygosity of an individual will be maintained during its multiplication.

The development of sources of genetic variability is a major challenge for breeders of apomictic species, particularly obligate apomicts (Bashaw, 1980). Essentially all of the seed produced by an obligate apomict and much of the seed from a facultative apomict are the result of asexual reproduction. The breeder must be able to obtain some level of sexual reproduction in the species to permit the recombination of genes from two or more different parents or to obtain

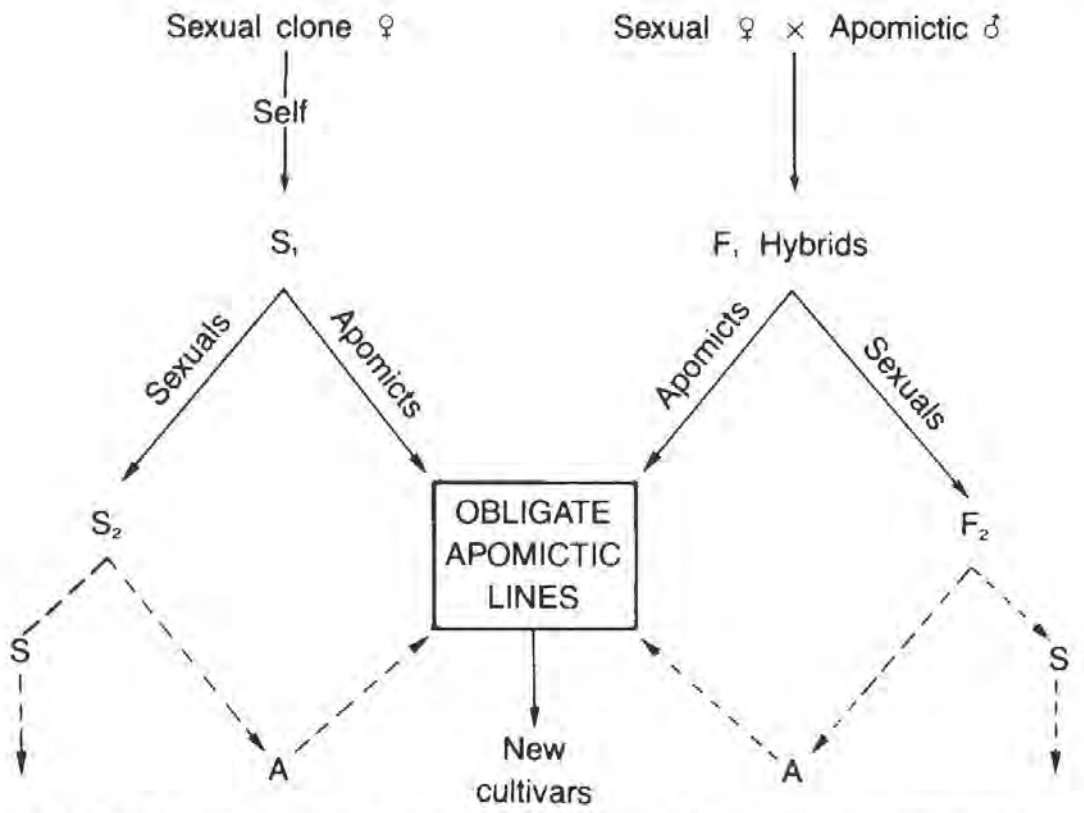


Figure 30-1 Methods used to obtain cultivars of buffelgrass, an obligate apomict. The sexual clone is heterozygous for genes controlling sexual reproduction, and sexual (S and F) and apomictic (A) individuals are found in the segregates obtained by self-pollination or hybridization. (Courtesy of Taliaferro and Bashaw, 1966.)

genetic variability by self-pollination of a heterozygous individual (Fig. 30-1). Apomixis is genetically controlled and can be manipulated by the breeder to some extent.

Several types of populations can be developed by artificial hybridization for the selection of superior clones.

Matings Between Current Cultivars

The most common method of population development is the crossing of current cultivars that have offsetting strengths and weaknesses. Because the cultivars are highly heterozygous, a cultivar × cultivar cross results directly in a new segregating population. The seed from the cross is planted and individual clones are evaluated as potential new cultivars.

The male parent of a cultivar × cultivar cross can be either known or unknown. Warner (1953) described a "melting pot" procedure for developing sugarcane populations in Hawaii. The females used in crosses were diverse, selected, male-sterile cultivars and the males were highly selected male-fertile cultivars.

The male parents were placed in the vicinity of females to permit cross-pollination.

Backcrossing

Modified backcrossing can be used to develop a population that includes a desired character from an otherwise unacceptable parent and the favorable characteristics of current cultivars (Fig. 30-2). The genotype of a heterozygous cultivar cannot be recovered by conventional backcrossing because offspring with homozygous loci are produced in the mating between heterozygous backcross progeny and the heterozygous recurrent parent. The homozygosity can result in undesirable inbreeding depression.

The conventional backcrossing procedure is modified by using a different desirable cultivar for each backcross (Fig. 30-2). The end result is a heterozygous population containing individuals with desirable characteristics from the recurrent parents and the desired trait from the donor parent. An individual may be identified from the population that is superior to the existing cultivars.

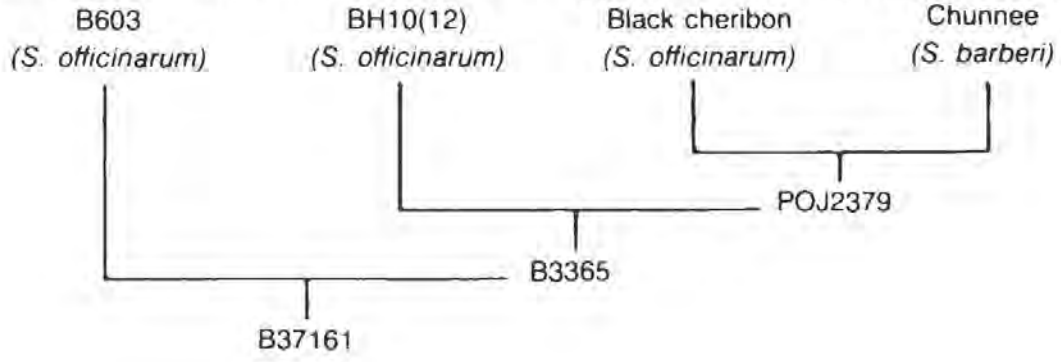
Recurrent Selection

Any of the methods of recurrent selection employed for seed-propagated species can be considered for population improvement in vegetatively propagated species. The method most commonly used is recurrent phenotypic selection (Chap. 15). As a population is improved, individuals with improved characteristics may be identified that can be utilized directly as cultivars.

Interspecific Hybridization

Hybrids obtained from the cross between heterozygous clones of different species represent a segregating population from which superior individuals may be iden-

Figure 30-2 Development of sugarcane cultivar by interspecific hybridization and modified backcrossing of *Saccharum* spp. (Courtesy of Stevenson, 1965.)



tified. Modified backcrossing also can be used to transfer to a segregating population a desirable character from a species that cannot be used effectively in a two-parent cross.

Interspecific hybridization has been used extensively in the breeding of sugarcane. Many modern cultivars have been derived from crosses of *Saccharum officinarum* with *S. spontaneum* or *S. barberi*. These crosses combined the high sugar content of *S. officinarum* with the disease resistance, cold tolerance, and vigor of *S. spontaneum* and *S. barberi*. A pedigree outlining an example of sugarcane cultivar development using interspecific hybridization and modified backcrossing is provided in Fig. 30-2.

Self-Pollination of a Cultivar

A vegetatively propagated cultivar is homogeneous because every propagule has the same genotype, in the absence of mutation. It is highly heterozygous, and self-pollination results in a segregating population that could be used for selection (Fig. 30-1). Self-pollination generally is not used for population formation, however, because inbreeding depression associated with homozygosity limits the value of the segregates as potential cultivars.

Matings Between Partially Inbred Parents

Cultivars have been developed from crosses between individuals with varying levels of inbreeding. The concept is the same as for the development of hybrid cultivars of seed-propagated species. Individuals in a segregating population are inbred, selection is practiced among inbred lines, selected lines are crossed, and the F_1 hybrids are evaluated. For vegetatively propagated species, superior hybrid clones can be used directly as cultivars.

The purpose of inbreeding is to eliminate deleterious recessive alleles that can be expressed only in the homozygous condition. Lines with unfavorable characteristics are discarded during inbreeding, and desirable lines are evaluated in hybrid combinations. There is considerable variability among species in their ability to tolerate inbreeding. There also are self-incompatibility systems that can limit self-pollination. For example, infertility caused by inbreeding leads to problems in breeding sugarcane. Many inbred lines of sugarcane have reduced vigor and fail to produce tassels. The selective breeding of inbred lines for the genetic potential of tassel production increases the frequency of tasseling in breeding material. However, increased tasseling is an undesirable character for cultivars that are used in a two-year sugarcane cropping system (Warner, 1953).

EVALUATION OF INDIVIDUALS

The evaluation of an individual can be done on the basis of a single plant, an unreplicated clonal plot, or a replicated clonal test. Because a superior clone is multiplied vegetatively as a cultivar, the breeder does not need to evaluate the genotype of an individual through some method of progeny evaluation.

The evaluation of clones for apomictic cultivars includes assessment of the level of asexual reproduction. Sexual reproduction is required to obtain genetic recombination for population development, but only segregates that reproduce by apomixis are considered as potential cultivars (Fig. 30-1). Cytological tests can be used to determine the mode of reproduction of an individual (Bashaw, 1980). Progeny tests of apomictic individuals result in a uniform group of plants, whereas sexual individuals will have segregating progeny (Fig. 30-3).

The genetic potential of clones can be evaluated as single plants or in clonal plots. Single-plant selection is useful for characters with high heritability that are not strongly influenced by the environment. The use of unreplicated clonal plots and replicated clonal tests is required for quantitative characters with moderate or low heritability.

Figure 30-3 Heterogeneous progenies from one sexual plant of buffelgrass are on the left of the photo and uniform progenies from obligate apomictic plants are on the right. (Courtesy of Bashaw, 1980.)



An example of a selection scheme for an asexually propagated species was described by Martin (1978) for potato. Seed obtained by artificial hybridization and by natural crossing were planted in fields infested with *Streptomyces scabies* (Thaxt.) Waks and Henric. Tubers produced on the plants were harvested in bulk without maintaining the identity of the plant from which they originated. A stepwise process of evaluation was conducted to identify superior tubers:

1. The tubers were washed and passed through a salt bath, and those with low specific gravity, growth cracks, malformations, or scab lesions were discarded.
2. Tubers that met the first criterion were stored on screens at 10 to 15°C and low relative humidity until tubers with poor storability could be eliminated.
3. The tubers that remained after the storage test were cut, and those with internal disorders were discarded.

The selection intensity used for evaluation by Martin depended on the number of tubers produced on plants grown from seed and on the number that could be handled at each phase of the screening program. The goal was to select 5000 to 8000 tubers for planting the next season in the field. Martin indicated that clonal tests were used to evaluate each selected tuber (clone) for potential value as a cultivar.

The size of plots, spacings, and cultural practices are unique for each species in which clonal evaluation is used. For example, Burton (1947) used several different procedures for clonal evaluation of bermuda grass in the development of the cultivar 'Coastal.' He selected 128 plants from 5000 that were grown in the field and used clonal tests to evaluate the selections.

Step 1: Clones from the 128 seedlings were planted in triplicate in pots in the greenhouse and evaluated for various characters.

Step 2: The plants in each pot were planted in the center of a 1.2-m × 7.3-m plot in the field. There were three pots of each clone from the greenhouse; therefore, there were three replicates of each clone in the field. The plants in the center of the plot spread by stolons until the entire area was covered. The important characteristics for the species were measured on the plots during 8 years.

Step 3: After 2 years of evaluation in the plots of step 2, two new trials were planted. Vegetative propagules were used to plant the plots.

1. Five of the best clones were planted in two replicates of plots 405 m^2 in size. The plots were evaluated for yield and chemical composition with and without fertilizer.
2. Nine clones were planted in two replicates of 9.1-m × 18.2-m plots for grazing trials. The palatability of each clone was determined by observing the feeding preferences of cattle.

Step 4: Two years after the plots in step 3 were established, nine clones were planted in 1.8-m × 5.5-m plots with four replications. The plots were

evaluated for response to close grazing by frequent cutting with a power mower.

COMMERCIAL PROPAGATION

One of the primary concerns in vegetative propagation of a cultivar is prevention of contamination by disease organisms and elimination of off-types that may arise by mutation. Virus infection is of particular concern in many species, and special nurseries have been established for the production of virus-free propagules.

For commerical production of obligate apomicts, the primary consideration is to prevent mechanical mixing of seed with seed of other cultivars during harvest. The absence of sexual reproduction eliminates the need for isolation to prevent outcrossing.

Cultivars of facultative apomicts have some heterogeneity due to the occurrence of a limited amount of sexual reproduction. The breeder must characterize the type and extent of variability in the cultivar. Environmental conditions may influence the mode of reproduction; therefore, genetic variation within the seed of a cultivar should be determined under the conditions in which commercial seed will be produced (Bashaw, 1980).

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CHAPTER THIRTY-ONE

Development of Self-Pollinated Cultivars

The self-pollinated cultivars grown by farmers throughout the world range from landraces that are heterogeneous mixtures developed by mass selection in natural populations to homogeneous genotypes developed by inbreeding and selection within populations formed by artificial hybridization or artificial mutagenesis. The origin of cultivars and their method of development can be reviewed by observing the stepwise process that occurs when a crop is introduced for commercial production into a country where it was not previously grown.

CROP INTRODUCTION

The first step in production of a new crop is to introduce cultivars from countries where they are currently grown. Cultivars may be heterogeneous if they originate from geographical areas where the crop has been grown for a long time, if farmers use their own seed to plant the crop each season, and if a high degree of uniformity is not required for commercial production. A higher degree of homogeneity generally would be present if the introduced cultivars had been developed by a plant breeder.

Introduced cultivars with adequate uniformity are evaluated for productivity, quality, and other characters considered important. The best ones can be released to farmers under the name used in the country from which they originated, or with another suitable designation.

SELECTION WITHIN HETEROGENEOUS CULTIVARS

Introduced cultivars that have good performance but are too heterogeneous can be subjected to individual plant selection followed by progeny testing. If plants

in the heterogeneous cultivars are homozygous, a series of homogeneous lines with different characteristics may be identified. Homogeneous lines with desirable performance can be released for use by farmers.

HYBRIDIZATION OR ARTIFICIAL MUTAGENESIS

After the genetic variability of germplasm from other countries has been exploited, the breeder must produce additional variability for the development of cultivars superior to those available. The development of a self-pollinated cultivar by use of hybridization or artificial mutagenesis involves four steps: (a) formation of a segregating population, (b) inbreeding the population by self-pollination to an adequate level of homozygosity, (c) evaluation of homogeneous lines and selection of the superior ones, and (d) preparation of seed stocks for commercial distribution of the cultivar.

Population Formation

The two ways of forming a segregating population are by hybridization and artificial mutagenesis. Both ways have been used to develop self-pollinated cultivars. Hybridization, however, is much more widely used than mutagenesis. A discussion of the relative merits of hybridization and mutagenesis is provided in Chap. 20.

The populations developed by hybridization include two-parent, three-parent, four-parent, and more complex crosses (Chap. 12). Backcrossing is used to form a segregating population and to transfer characters from one cultivar to another (Chaps. 12 and 28). Populations improved by recurrent selection can be used to develop self-pollinated cultivars (Chaps. 15 and 16).

Self-Pollination to an Adequate Level of Homozygosity

Two of the major decisions that must be made by the breeder are the number of generations of inbreeding that will be conducted in a population before plants are selected for evaluation as potential new cultivars and the method of managing the population during inbreeding. The variation in strategies used by breeders to develop cultivars reflects the array of alternatives that can be considered in making the decisions.

Number of Inbreeding Generations. A self-pollinated cultivar is developed by selecting an individual plant, evaluating the bulk progeny as a breeding line, and releasing the superior line as a cultivar. Cultivars of self-pollinated species

are derived as early as the F_2 and and as late as the F_{11} generation. The generation in which a cultivar is derived refers to the generation of inbreeding of the single plant from which the cultivar originated. The generation in which cultivars are derived is dependent on the degree of uniformity required within a cultivar, the number of generations of self-pollination required to obtain an adequate number of lines with that level of uniformity, and the time required to develop and release a cultivar.

The level of uniformity required in a cultivar is determined by the breeder, seed regulatory agencies, farmers, and the consumer. The breeder must be confident that the genetic makeup of a cultivar will not change during multiple generations of seed production. A line derived in early generations that contains genotypes with differential competitive ability may undergo significant genetic changes during successive generations due to intergenotypic competition, in the same manner as a planned seed mixture of cultivars (Chap. 32).

Seed regulatory agencies, including plant variety protection and seed certification, have requirements for uniformity that must be considered (Chap. 36). Those agencies often are concerned about variability for qualitative characters, such as hilum and pubescence color of soybean and seed color of oat. A breeder may choose to discard heterogeneous lines before evaluation or may evaluate heterogeneous lines and purify any superior ones before their release as a cultivar.

Farmers may have preferences for uniformity of certain characteristics, particularly those that influence the harvestability and marketability of the crop. Uneven maturation or height of a crop can influence the effectiveness of mechanical harvest. There are, however, qualitative characteristics for which uniformity may not be important to the farmer. For example, hilum color of soybean seed is not a factor in marketing, and farmers generally are unconcerned about heterogeneity for the trait.

After the required level of uniformity has been determined, it is necessary to establish the number of selfing generations required to obtain a sufficient number of lines for evaluation that have adequate uniformity. The percentage of homozygous individuals in a population increases with each generation of self-pollination. It may be possible to obtain uniform lines from F_2 plants, but the percentage of such lines would be less than the percentage from later generations of inbreeding. Breeders who develop cultivars derived from F_2 and F_3 plants must grow a larger number of progenies to obtain a given number of uniform lines than breeders who delay selection until later generations.

The generation in which lines are derived from a population can influence the time required to develop and release a cultivar. If a breeder can grow only one generation a year, each generation of selfing that takes place before lines are derived adds one additional year to the length of cultivar development. However, for annual species the ability to grow populations in off-season nurseries and greenhouses often permits an increase in the number of generations of inbreeding without increasing the time required for cultivar development.

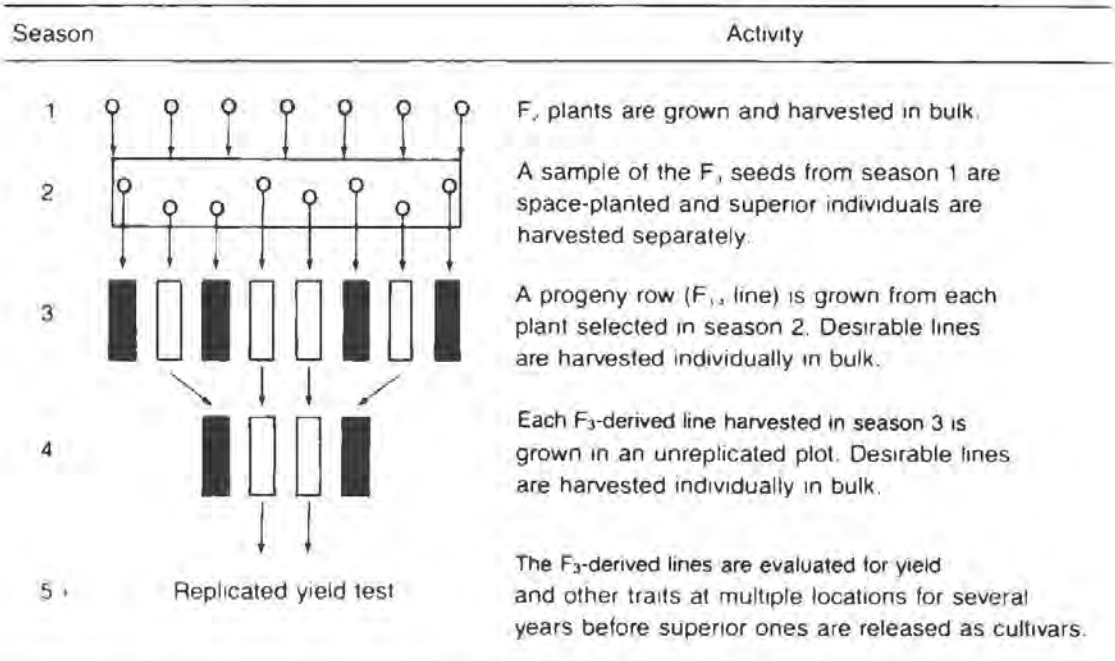


Figure 31-1 Outline of a procedure used by Ohio State University, Wooster, to develop by the bulk method winter wheat cultivars derived from F_3 plants. Dark rectangles represent discarded lines.

Methods of Inbreeding. The basic methods of managing a population during inbreeding include pedigree, bulk, single-seed descent, early-generation testing, and mass selection. These methods are used individually and in various combinations by breeders of self-pollinated species for the development of improved cultivars. Examples of methods currently in use are given in the following descriptions and in Figs. 31-1 and 31-2.

Outline of Procedure Used at Iowa State University, Ames, to Develop by the Multiple-Seed Procedure of Single-Seed Descent Soybean Cultivars Derived from F_4 Plants.

Season 1: Two-hundred fifty F_2 seeds of a population are planted about November 1 in Puerto Rico under natural day length conditions. After about 90 days, one three-seeded pod is harvested from each plant and the pods are threshed in bulk. Two-hundred fifty seeds are used for season 2 and the remainder are kept as a reserve.

Season 2: Two-hundred fifty F_3 seeds are planted about February 1 in Puerto Rico under natural day length conditions. After about 90 days, one three-seeded pod is harvested from each plant and the pods are threshed in bulk. Two-hundred fifty seeds are used for season 3 and the remainder are kept as a reserve.

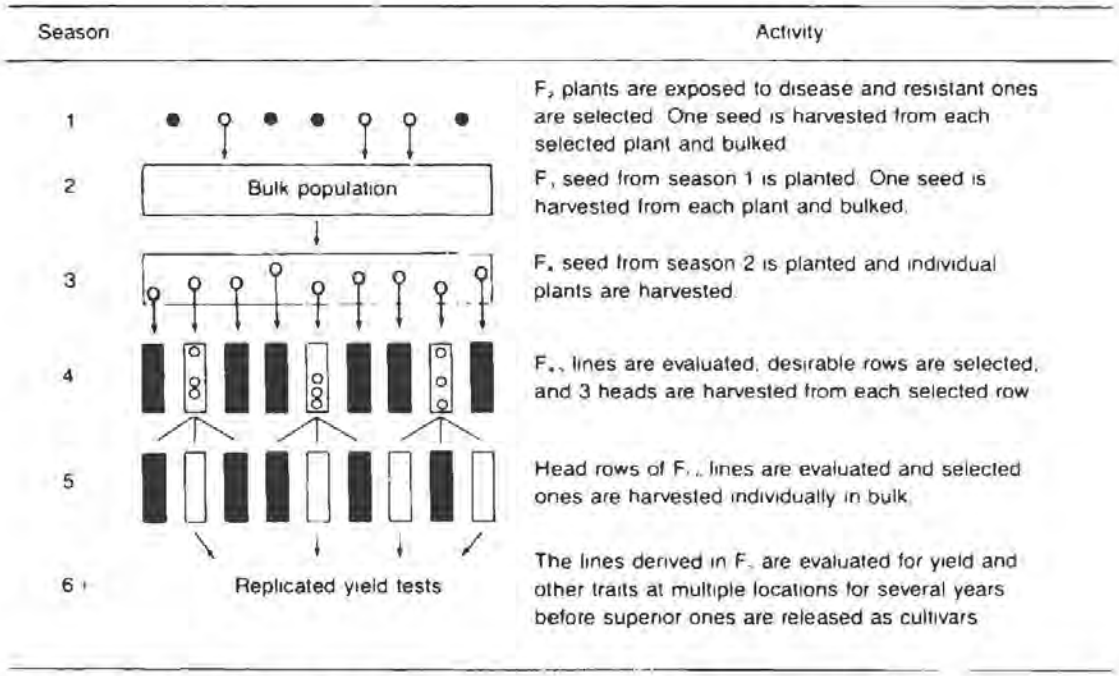


Figure 31-2 Outline of a procedure used by the University of Minnesota, St. Paul, to develop by mass selection, single-seed descent, and pedigree selection spring wheat cultivars derived from F_5 plants. Dark circles and rectangles represent discarded plants or lines.

Season 3: Two-hundred fifty F_4 seeds are planted in May in the northern United States, and individual plants are harvested.

Season 4+: The lines derived in F_4 are evaluated for yield and other traits at multiple locations for several years before superior ones are released as cultivars.

Outline of Procedure Used by Ohio State University, Wooster, to Develop by Early-Generation Testing Soybean Cultivars Derived from F_4 Plants.

Season 1: F_2 plants are grown, and desirable ones are harvested individually.

Season 2: Progeny of each plant harvested in season 1 ($F_{2:3}$ lines) are evaluated for yield in an unreplicated plot.

Season 3: The highest yielding lines in season 2 are evaluated for yield as $F_{2:4}$ lines in replicated plots. Individual F_4 plants are harvested from the border rows of a plot of each line.

Season 4+: Progeny from individual F_4 plants (F_4-derived lines) harvested from the superior $F_{2:4}$ lines in season 3 are evaluated for yield and other traits at multiple locations for several years before superior ones are released as cultivars.

Outline of Procedure Used by the University of Wisconsin, Madison, to Develop by Pedigree Selection Oat and Spring Wheat Cultivars Derived from F_5 Plants.

Season 1: F_2 plants are grown, and desirable ones are harvested individually.
Season 2: A progeny row ($F_{2:3}$ line) is grown from each plant selected in season 1. Five or six plants are harvested individually from selected rows.
Season 3: A progeny row ($F_{3:4}$ line) is grown from each plant harvested in season 2. Five or six plants are harvested individually from selected rows.
Season 4: A progeny row ($F_{4:5}$ line) is grown from each plant harvested in season 3. Five or six plants are harvested individually from selected rows.
Season 5: A progeny row ($F_{5:6}$ line) is grown from each plant harvested in season 4. Desirable rows are harvested individually in bulk.
Season 6+: The lines derived in F_5 are evaluated for yield and other traits at multiple locations for several years before superior ones are released as cultivars.

Outline of Procedure Used by Oklahoma State University, Stillwater, to Develop Winter Wheat Cultivars Derived from F_2 Plants.

Season 1: F_2 plants of a population are grown, and desirable ones are harvested individually.
Season 2: $F_{2:3}$ lines are evaluated in unreplicated plots and the most desirable ones are harvested individually in bulk.
Season 3: $F_{2:4}$ lines are evaluated in unreplicated plots and selected ones are harvested individually in bulk.
Season 4+: The lines derived in F_2 are tested for yield and other traits at multiple locations for several years before superior ones are released as cultivars.

Outline of Procedure Used by Virginia Polytechnic Institute and State University, Blacksburg, to Develop by Mass Selection with Self-Pollination and the Bulk Method Barley Cultivars Derived from F_5 Plants.

Season 1: F_2 plants are grown, desirable individuals are selected, and selected plants are threshed together in bulk.
Season 2: A sample of F_3 seed from season 1 is planted. Desirable individuals are selected and threshed together in bulk.
Season 3: A sample of F_4 seed from season 2 is planted. Desirable individuals are selected and threshed together in bulk.
Season 4: A sample of F_5 seed from season 3 is planted. Desirable individuals are harvested individually.
Season 5: A progeny row ($F_{5:6}$) is grown for each plant selected in season 4. The superior ones are harvested individually in bulk.

Season 6+: The lines derived in F_5 are evaluated for yield and other traits at multiple locations for several years before superior ones are released as cultivars.

Outline of Procedure Used by the University of Minnesota, St. Paul, to Develop by Pedigree Selection Oat Cultivars Derived from F_3 Plants.

Season 1: F_2 plants of a population are exposed to disease, and desirable plants are harvested individually.

Season 2: $F_{2:3}$ lines are evaluated in unreplicated plots, and five panicles are harvested from each selected row. The remainder of the row is harvested in bulk and the seed analyzed for protein content. Panicles are retained from rows with adequate protein composition.

Season 3: A progeny row ($F_{3:4}$ line) is grown from each panicle selected in season 2. Superior lines are harvested individually in bulk.

Season 4+: F_3-derived lines are evaluated for yield and other traits in multiple locations and years before superior ones are released as cultivars.

The method chosen to manage a population is strongly influenced by the length of time a breeder is willing to spend in the development of a new cultivar and the suitability of available environments for selection and replicated testing. The pedigree method and mass selection with self-pollination can only be used in environments where selection for the characters of interest is possible. The bulk method is not suited to environments in which natural selection is likely to favor undesirable genotypes. The replicated testing phase of the early-generation method must be done in environments where characters can be measured appropriately. Only single-seed descent can be used in any environment, regardless of its suitability for artificial or natural selection.

Breeders often vary the method of inbreeding to maximize the use of available environments. This flexibility was emphasized by Harrington (1937) when he proposed the use of the mass-pedigree method of inbreeding. He had found that selection in the F_2 generation for straw strength, resistance to some diseases, plant height, earliness, and shattering resistance was largely ineffective in dry seasons. The effectiveness of selection in later generations also was reduced under these conditions, although to a lesser extent. Utilization of the typical pedigree method proved expensive and thus constituted a large loss of resources in dry seasons. The strict use of mass selection, however, resulted in smaller gains during favorable years than use of the pedigree method. A modified method was developed to overcome the limitations imposed by the frequent occurrence of dry seasons.

> The modifications consisted in selecting for desirability in one or more important characters when circumstances were favorable. A wet season or a combination of long straw and high winds sometimes presented an excellent opportunity for selection for resistance to lodging as well as for several other characters. Such opportunities were taken advantage of, whether they occurred when a cross was in F_2 or in any

later segregating generation. Again, if a satisfactory disease epidemic occurred naturally, or was induced artificially, selection for resistance was made in the mass plats, irrespective of the generation they were in. In very dry or hot seasons, selection for drought and heat resistance was carried on.

These modifications led to the introduction of the progeny test as a further feature of the method and owing to this feature the process was called the "Mass-Pedigree Method." The plan is to go on with individual plant progeny tests whenever the circumstances have particularly favored selection in the preceding year. [Harrington, 1937]

The mass-pedigree method is just one of several possible combinations of inbreeding methods. The procedure outlined in Fig. 31-2 includes mass selection with self-pollination, single-seed descent, and pedigree selection. By fitting the inbreeding method to the environment, it may be possible for the breeder to practice selection in some generations without increasing the length of time for cultivar development.

Selection of a method for inbreeding a population can have an impact on the genetic improvement per year realized from a breeding program. Many of the concepts for maximizing genetic gain that are associated with methods of recurrent selection apply equally well to methods of inbreeding. A breeder will consider those concepts when planning an appropriate strategy for inbreeding (Chap. 17).

Evaluation of Lines

The evaluation of homogeneous lines in replicated tests is a major aspect of any breeding program. The breeder must decide if a line will have the desired performance for quantitative characters over a range of environments. The number of locations and years of replicated testing that are conducted before a homogeneous line is released as a cultivar generally is not influenced by the method of inbreeding used. The primary difference among methods is the division of resources between tests conducted during inbreeding and those available for the evaluation of homogeneous lines. Every breeder has a fixed amount of resources available to carry out all phases of selection and testing; therefore, resources expended during the inbreeding program will reduce those available for evaluation of homogeneous lines in replicated tests.

The most obvious division of testing resources is for early-generation testing. Heterogeneous lines or populations are evaluated in replicated tests, and the inferior ones are discarded. Every plot used to evaluate a heterogeneous line or population reduces by one the number available to evaluate homogeneous lines. Therefore, the number of homogeneous lines that can be evaluated with early-generation testing is less than with the other methods of inbreeding. The division of resources between the two phases of testing is a major decision for the breeder. Those who use the method believe that use of testing resources in early gener-

ations is worthwhile to improve the sample of homogeneous lines available in later generations of selection.

Pedigree selection does not utilize resources for replicated testing per se, but an extensive amount of time and land is needed for evaluation during inbreeding. The time and land used for selection during inbreeding could be spent instead to evaluate more homogeneous lines in a replicated testing program. Breeders who use the pedigree method are willing to expend resources during inbreeding so that the homogeneous lines entered into replicated tests are superior to a group of random lines for highly heritable characters.

The mass selection, bulk, and single-seed descent methods do not involve any progeny testing during inbreeding. As a result, more resources are available for replicated testing of homogeneous lines than for the early-generation testing or pedigree methods. Homogeneous lines obtained from mass selection, bulk, or single-seed descent generally are evaluated during one season for the same highly heritable characters considered in pedigree selection, and the superior ones are entered into replicated tests.

The emphasis in the first season of replicated testing is to evaluate as many lines as possible in a limited number of locations and replications per location. In the following seasons, the number of lines is progressively decreased and the number of locations is progressively increased. The plot size and type also may be changed during the different testing phases (Chap. 19).

Preparation of Breeder Seed

When a new cultivar is developed, the breeder prepares a seed sample that is used for increasing seed of the cultivar for distribution to farmers. The sample commonly is referred to as breeder seed because the breeder generally is responsible for producing and maintaining it. It also is called basic seed because it represents the genetic basis of the cultivar from which all subsequent seed is produced. The breeder seed is used to produce foundation seed. The foundation seed and subsequent generations of production generally are produced on a large scale by persons other than the breeder (Fig. 31-3).

The preparation of breeder seed generally begins before the decision to release a line has been made, which requires extra effort by the breeder because seed of lines not released must be discarded. However, simultaneous line evaluation and breeder seed production reduces the length of time required to have seed of a new cultivar available to farmers. The following procedure has been used by the soybean breeding project at Iowa State University. After 2 years of testing in Iowa, about 1 percent of the lines first entered in the yield evaluation program have been selected. During the third to fifth years, the lines are evaluated extensively in states of similar latitude. If a line is selected for release, breeder seed is available for immediate production of foundation seed.

Some breeders prefer to begin the preparation of breeder seed the first or

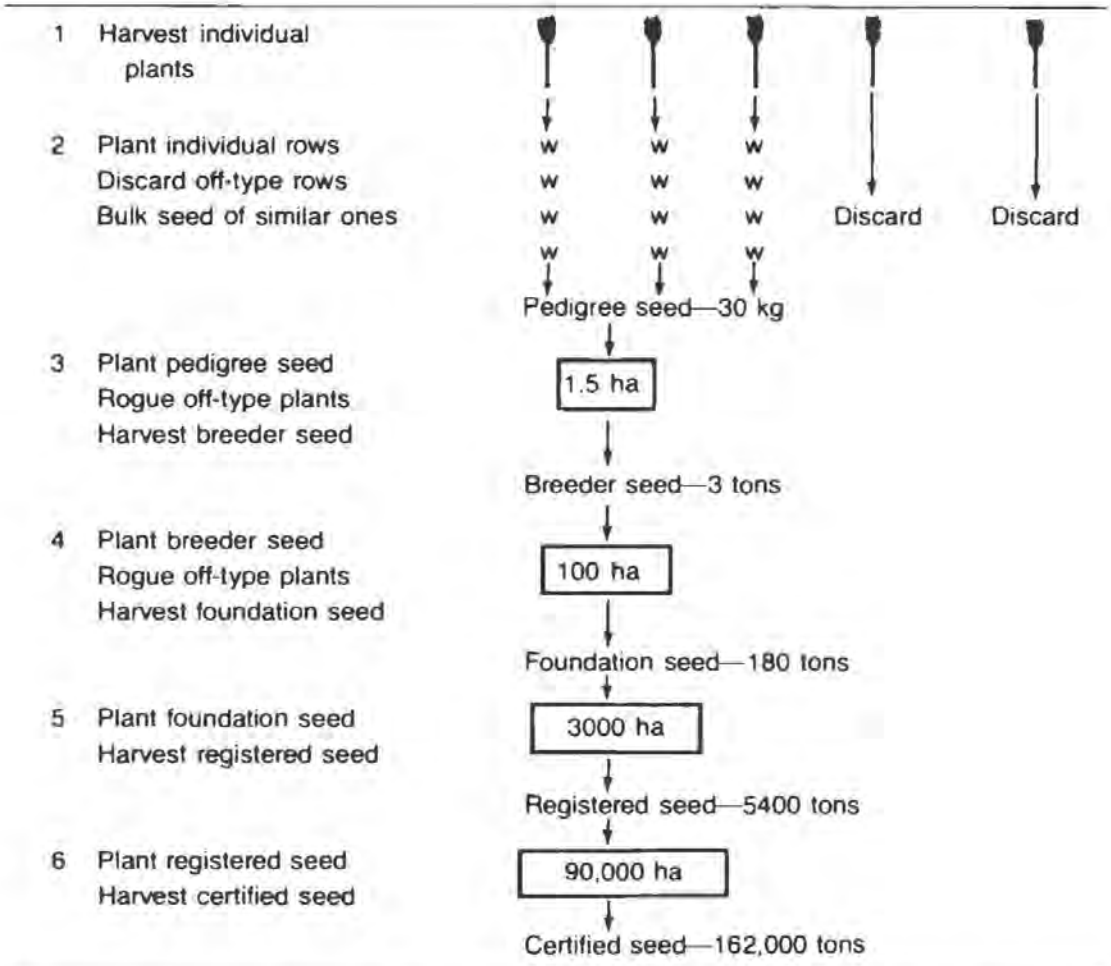


Figure 31-3 Illustration of a procedure for purification and seed increase of a new cultivar. The kilograms, tons, and hectares indicated are approximate quantities that could be produced for each seed class. (Courtesy of Fehr, 1978.)

Line Evaluation	*Seed Multiplication*
First year	—
Second year	—
Third year	Single plants harvested
Fourth year	Progeny rows grown
Fifth year—Decision to release is made	Progeny rows increased and bulked to form breeder seed

second year a line is tested. This reduces the time required to make large quantities of seed available to farmers, but increases the cost of seed stock preparation because many more lines must be handled.

The seed used to develop breeder seed can be derived from yield tests in which mechanical mixing is minimized or from a seed sample of the line that was retained in storage. Some breeders put a seed sample of each line in storage when replicated tests begin. The seed from storage is used to produce breeder seed of a line chosen to be a cultivar, instead of seed obtained from replicated tests.

Initial Production of Breeder Seed. The two alternatives for the initial production of breeder seed are mass selection and progeny testing.

Mass Selection. For mass selection, a sample of seed with uniform characters is planted, off-type plants are removed before harvest, and the remaining plants are harvested in bulk to obtain breeder seed. The advantages of the procedure are that breeder seed can be produced in one season and that the procedure requires much less time and expense than the use of progeny testing. The disadvantage is that a high degree of uniformity is difficult or impossible to achieve. Individual off-type plants are more difficult to identify than a group of off-type plants in a progeny row, particularly for quantitative characters. Heterozygous and homozygous plants cannot be distinguished for characters controlled by dominant alleles.

Progeny Testing. The use of progeny testing provides the greatest assurance of achieving a high degree of uniformity in the breeder seed. Steps are as follows:

Season 1: A sample of seed with uniform characteristics is planted. Plants with similar phenotypic characteristics, such as flowering date, color of plant parts, and leaf size and shape, are selected and threshed individually. The seed from each plant is inspected, and plants with similar seed characteristics are retained.

Season 2: Plants from season 1 are progeny tested for important characteristics, such as pest resistance and agronomic traits. Rows that are segregating for any important characteristic or that are dissimilar from the majority of the other rows are discarded. Each selected row is harvested separately. The seed from each row is inspected for uniform characteristics.

Season 3: Seed from each row in season 2 is grown in a separate increase block, and the plants are inspected for uniformity. Blocks that are not uniform are discarded. The selected blocks are harvested in bulk and properly mixed to obtain breeder seed.

The second progeny test in season 3 frequently is omitted—uniform families in season 2 are harvested together in bulk and properly mixed to obtain breeder seed. Elimination of the second progeny test in season 3 reduces the number of

seasons and the expense required, but does not ensure the same level of genetic purity.

The number of progenies that are evaluated and mixed together to produce breeder seed varies widely among breeders. Some are satisfied to use only a few progenies, while others prefer to maintain possible heterogeneity for nonvisual characters by including the progenies of 100 or more plants.

The advantage of progeny testing is that a high degree of uniformity can be achieved in the breeder seed. The disadvantages are that two or more seasons are required and much more time and expense are involved than with the use of single-plant selection.

Repeated Production of Breeder Seed. Several procedures are used to provide an adequate supply of breeder seed each year for established cultivars.

1. A quantity of breeder seed is put in storage when the cultivar is first released. When the supply of seed in storage becomes inadequate, a new supply is developed by the progeny-testing procedure. This method is effective if adequate storage facilities are available to maintain the required seed supplies.
2. Breeder seed is produced each season by progeny testing. The method is effective in providing pure seed each year, but can require considerable time and expense when a large number of cultivars are involved.
3. Part of a foundation seed field is designated for use in obtaining breeder seed. The area is rogued rigorously for off-type plants and harvested separately from the remainder of the field. The procedure is continued until the frequency of off-types that cannot be identified on a single-plant basis becomes too high, at which time the cultivar is purified by progeny testing. The method provides a supply of breeder seed of adequate genetic purity at a lower cost than alternative 2, but the genetic purity for all characters may be less.

Examples of Breeder Seed Preparation. Mass selection is used to obtain breeder seed of winter wheat and oat by the University of Wisconsin, Madison. Seed obtained from yield tests of a potential new cultivar are sown in a plot 3 m × 25 m. Off-type plants are rogued for maturity and height, and the plot is harvested in bulk as breeder seed.

Progeny testing for one year is used to obtain breeder seed of spring wheat by the University of Minnesota, St. Paul. Five-hundred heads are harvested individually from a potential new cultivar. A progeny row is grown from each head, and rows with uniform characteristics are harvested in bulk as breeder seed.

The Foundation Seed Organization of the University of Nebraska maintains breeder seed of wheat by marking out an area in a foundation seed field, roguing

the area carefully, and harvesting the rogued area separately from the remainder of the field.

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CHAPTER THIRTY-TWO

Multilines

Planned seed mixtures of different genotypes are an alternative to cultivars that are an individual pure line, hybrid, or clone. Mixtures are used commercially in such self-pollinated species as oat, soybean, wheat, and peanut. In turfgrasses, both intraspecies and interspecies mixtures are widely used. Mixtures of hybrid cultivars are theoretically possible but have not been employed to date.

Mixtures of seeds of different genotypes are referred to as multilines and blends. The terms are used interchangeably, even though some persons prefer multilines to represent mixtures of isolines and blends to designate mixtures of lines differing for multiple characters.

The value of heterogeneity in crop cultivars has been discussed for many years. Several levels of heterogeneity and approaches to the development of heterogeneous mixtures have been proposed in species propagated by seed. A high level of diversity was suggested by Rosen (1949) for control of crown rust and *Helminthosporium* blight of oat. He suggested the commercial use of heterogeneous populations obtained by artificial hybridization, rather than of homogeneous cultivars. One mixed population released for commercial use was 'Harland' barley. Suneson (1968) described it as a population cultivar because it was a composite of crosses maintained in bulk for many generations. The concept was to release the heterogeneous mixture and allow it to undergo natural selection during seed production. Suneson indicated that the heterogeneous cultivar should improve continually over generations if natural selection favors high-yielding individuals.

The second suggestion for obtaining heterogeneity was proposed by Jensen (1952). He suggested the use of mixtures of cultivars or lines with similar phenotypes for intravarietal diversification of oat. Mixtures of cultivars are used commercially in soybean and oat in the United States. A mixture of closely related lines of wheat, 'KSML3,' was released in India to provide improved disease resistance (Gill et al., 1980).

The third approach to multiline development was proposed by Borlaug (1959). His plan was to prepare cultivars that were a seed mixture of a number of phenotypically similar lines developed by backcrossing. Each line in the mixture would possess different genes for disease resistance. Mixtures of isolines of oat have been released in Iowa (Frey et al., 1975), a mixture of wheat isolines was released under the name of 'Tumult' in the Netherlands (Groenewegen, 1977), and a mixture of wheat isolines was released in Colombia as 'Miramar 63'.

PURPOSE OF MIXTURES

Pest Control

Seed mixtures were suggested by Rosen (1949) as a means of minimizing loss from pests that have multiple races whose frequencies can shift from year to year. The probability that all plants of a heterogeneous mixture would be severely damaged by a pest is less than that for a homogeneous cultivar. The mixture can be considered insurance against severe crop loss.

There is a disagreement among plant pathologists about the ability of a mixture to influence the race structure of the pest from year to year. Such an influence could occur if the pest population at the end of one year were the breeding pool for the pest population the following year.

A mixture of lines with different genes for resistance to a wind-borne disease can delay spread of the pathogen within a field (Browning and Frey, 1969). The delay is associated with the inability of the pathogen to reproduce on resistant plants. The resistant plants serve as a trap and minimize the number of spores available for infection of susceptible individuals (Chap. 21).

Marketing

A mixture can be a useful marketing aid for a seed merchandiser. In the United States, a mixture of two or more cultivars or species can be sold under any brand name if a label is attached that reads "Variety not stated." The same mixture can be sold by two or more seed merchandisers under different brand designations. For example, a mixture of two oat cultivars in a 1:1 ratio could be sold as William Brand, Henry Brand, or Milton Brand.

Adaptation to Different Environments

One advantage of mixtures that is commonly cited is their adaptation to different environments. A blend of turfgrass seed provides a means of obtaining an appropriate ground cover under an array of environmental conditions. In the north

central United States, a mixture of ryegrass and several apomictic cultivars of Kentucky bluegrass are sold commercially. The ryegrass has rapid stand establishment and does better than bluegrass in shaded areas. Use of several bluegrass cultivars provides some assurance that at least one will be adapted to an environment.

Seed mixtures generally are considered to exhibit less fluctuation in performance across environments than homogeneous cultivars. This is one of the reasons for the development of heterogeneous peanut cultivars for the southern United States (Norden, 1980).

Minimization of the Impact of a Deficiency in a Cultivar

There are situations in which the most highly productive cultivar available is vulnerable to a production hazard that occurs sporadically. A mixture of the high-yielding, susceptible cultivar and a lower-yielding, resistant cultivar may be useful during the period required to develop a high-yielding, resistant cultivar. Yield of the blend would be less than that of a pure stand of the productive cultivar in the absence of the problem, but could be considerably greater when the problem is present.

DEVELOPMENT OF MULTILINES

The procedure used for the development of multilines depends on the type that is used commercially. The types of multilines are a mixture of isolines, closely related lines, or distinctly different genotypes.

Mixtures of Isolines

Mixtures of isolines have been used exclusively as a strategy for pest control. Isolines for a mixture are developed by transferring different genes for pest resistance into one recurrent parent by backcrossing. The genes are transferred independently in separate backcrossing programs to obtain a series of backcross-derived lines that are the same except for the genes controlling resistance. Seeds of each of the isolines are multiplied separately, then mixed together in the desired proportions for commerical plantings.

In the strict sense, isolines have identical genotypes except for genes controlling one character. True isolines are difficult, perhaps impossible, to achieve with conventional hybridization procedures because of linkage between the gene of interest and those influencing other characters. The transfer that occurs with backcrossing involves a block of closely linked genes instead of a single gene.

Conventional backcrossing procedures are used to develop the isolines. Several factors are considered in initiating programs for isoline development.

Selection of the Recurrent Parent. A line derived by backcrossing generally will not be superior to the recurrent parent except for the character being transferred. This principle is extremely important in the selection of the recurrent parent for developing a series of isolines. The recurrent parent should be the best cultivar or line available for traits of major economic importance. Because no perfect cultivar exists for all agronomic characters, the breeder generally must choose the one with the fewest weaknesses.

Selection of the Donor Parents. The nonrecurrent parents should be lines that are resistant to as many known races of the disease as possible. For stem rust resistance in wheat, Borlaug (1959) examined stem rust reactions of cultivars included in the Co-operative International Stem Rust Nursery. Some cultivars in these nurseries were resistant throughout the world and were chosen as donor parents.

Donor parents commonly are unadapted genotypes. For oat multiline development, introductions of *Avena sterilis* have been used to obtain resistance genes to crown rust (Frey et al., 1975). *A. sterilis* is a weedy oat of no commerical value.

The resistance in a donor parent is determined by evaluating its response to a number of races of the organism. Genotypes with different reactions to different races are assumed to have different genes controlling resistance. Consider the hypothetical case of nine potential donor parents in Table 32-1 that were tested with 12 races. The reactions were either resistant (R), moderately resistant (MR),

Table 32-1 Reaction of Nine Genotypes (Potential Donor Parents) to 12 Hypothetical Races of a Disease Organism

	Race											
Genotype	101	102	103	104	105	106	107	108	109	110	111	112
1	MR*	R	MR	R	R	MS	MR	MR	S	R	MS	MR
2	R	R	R	R	R	S	R	MS	MR	R	R	MS
3	R	R	R	R	R	R	R	R	R	R	MR	MS
4	R	R	R	MR	R	S	S	S	MS	MS	S	S
5	R	R	R	R	R	R	MR	S	MR	R	MS	MS
6	MR	R	R	MR	R	S	MR	MR	MR	R	S	MR
7	MR	R	R	MR	MS	MR	MS	MS	MS	MS	S	MR
8	R	R	R	R	R	R	R	R	MS	R	R	MR
9	R	R	R	R	R	R	R	R	MS	R	R	MR

*R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

moderately susceptible (MS), or susceptible (S). All of the genotypes had different reactions, except 8 and 9. Because 8 and 9 had the same reactions, they would be assumed to have the same genes for resistance, and probably only one of the two lines would be chosen as a donor parent.

Borlaug (1959) used as donor parents several genotypes that were completely resistant to all known races of stem rust. There would be no way of knowing if the genotypes possessed the same genes for resistance until a race developed that attacked them differentially.

Evaluation for Disease Resistance During Backcrossing. Isolines have different nonrecurrent parents and are developed by independent backcross programs that take place concurrently. Presumably the genes for resistance in all the isolines will be different. The only way to be certain that the genes from the nonrecurrent parent are being transferred is to test every plant with all the disease races needed to characterize its resistance. Such a screening procedure would be extremely difficult to accomplish.

To reduce the work required to evaluate for resistance, a single tester race commonly is used for as many isolines as possible. The tester race is one to which the recurrent parent is susceptible and the nonrecurrent parents are resistant. For example, if the recurrent parent is susceptible to race 275 and the nonrecurrent parents are resistant, then race 275 could be the tester race.

The tester race is used until the final backcross is completed. The selections from each isoline backcross program generally are tested with a wide array of disease races before they are bulked to form the isoline. The final disease evaluation is to ensure that genes for resistance from the nonrecurrent parent were not lost during backcrossing.

Number of Backcrosses. The number of backcrosses used depends on (a) the need for the backcross lines to resemble the original recurrent parent, (b) the agronomic similarity between the recurrent and nonrecurrent parents, and (c) the amount of testing of the lines before commercial use. Three backcrosses generally are considered sufficient if individual lines in each backcross program are yield tested before they are bulked to form an isoline. At least four backcrosses generally are used if no yield testing is used before lines are bulked to form an isoline.

The multilines of oats developed in Iowa were derived from five backcrosses; and a multiline of wheat, 'Tumult,' involved six backcrosses.

Evaluation of Lines After Backcrossing. When the last backcross is complete, individual lines from each backcross program are evaluated. Desirable lines from each program are bulked to form an isoline that may be used to form a mixture.

The following is the procedure described by Borlaug (1959) for selecting lines for an isoline.

Season 1: Self BC_xF_1 plants from the last backcross.

Season 2: Grow a large BC_xF_2 population and subject it to the tester race. Select resistant plants with agronomic similarity to the recurrent parent. Characters with a high heritability, such as maturity and plant height, can be selected on an individual plant basis.

Season 3: Grow the progeny of each BC_xF_2 plant ($BC_xF_{2:3}$ lines) in an unreplicated plot in a disease nursery. Test each row for resistance with at least two different races, including the tester race used during backcrossing. Harvest rows that possess adequate resistance and resemble the recurrent parent for agronomic characters, such as maturity, height, and grain quality.

Season 4: Evaluate the $BC_xF_{2:4}$ lines in replicated yield trials and in a disease nursery. Select desirable lines for further testing.

Season 5: Evaluate selected $BC_xF_{2:5}$ lines in replicated yield trials and a disease nursery. Test the lines individually and when bulked together.

Season 6: Test superior $BC_xF_{2:6}$ lines against a wide range of disease races. Bulk lines with similar disease resistance to form an isoline.

The oat project at Iowa State University used less yield evaluation for selecting lines to put in an isoline (Frey et al., 1975).

Season 1: Self BC_xF_1 plants from the last backcross.

Season 2: Grow about 3000 BC_xF_2 plants in the greenhouse, inoculate with the pathogen, and select 200 to 600 resistant plants.

Season 3: Plant the seed from each BC_xF_2 plant in a hill plot in the disease nursery. Discard hills that are not homogeneous for disease resistance against the tester race. Discard hills that are off-type agronomically. Harvest about 125 to 150 lines individually in bulk.

Season 4: In the greenhouse, test each $BC_xF_{2:4}$ line against several disease races. Bulk about 100 $BC_xF_{2:4}$ lines to form the isoline.

Season 5: Yield test the isoline.

Preparation of the Multiline for Commercial Use. Each isoline is increased separately, then the isolines are mixed to obtain seed for commercial plantings. One possible chronology of seed production is as follows:

Season 1: Increase each isoline. Obtain enough seed (about 1.5 metric tons) to form the initial mixture and for use in future mixtures.

Season 2: Mix seed of selected isolines to form the multiline, plant it, and harvest foundation seed.

Season 3: Distribute foundation seed to commercial seed growers for increase. Seed produced by commercial seed growers is distributed to farmers.

The isolines chosen for the mixture and the proportion of each is based on the prevalent disease races. This necessitates a seasonal disease survey to monitor shifts in the disease population. In oat, Frey and colleagues (1975) indicated

Table 32-2 Reactions of Isolines Used in 'Multiline E68' to Races of Crown Rust Prevalent in United States in 1966

Isoline	Race*			Percentage of Isolines in Multiline E68
	216	290	326	
C237-89II	S	MS	MS	8
C237-89V	MR	MR	MS	8
X292II	MR	MR	MS	16
X434II	R	R	R	9
X467	R	MR	S	8
X468II	MR	MR	S	8
X469II	MR	MR	R	5
X469III	MR	MR	R	8
X470I	R	R	R	24
X466I	R	R	S	7

*Races 216, 290, and 326 represent three successive and overlapping stages of rust-race evolution. R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.
Source: Frey et al., 1971.

that at least 60 percent of the mixture should be resistant to prevalent disease races. The frequency of each isoline does not have to be equal, but no isoline should exceed 25 percent of the blend.

The mixture can be reconstituted as frequently as is needed to cope with new disease races. The composition of the mixture can be changed if superior isolines for disease resistance or agronomic characteristics are developed.

Mixtures developed in Iowa illustrate the percentage of isoline components used and the change in components over time (Frey et al., 1971). 'Multiline

Table 32-3 Reactions of Isolines Used in 'Multiline E70' to Races of Crown Rust Prevalent in United States in 1968

Isoline	Race*			Percentage of Isolines in Multiline E70
	290	325	264B	
C237-89V	MR	MS	S	5
X292II	MR	MS	MS	11
X434II	R	R	R	22
X467	MR	S	S	5
X468II	MR	S	S	5
X470II	R	R	S	21
X466	R	S	MS	5
B313-12	MR	S	S	5
X465	MR	S	S	5
X539III	MR	MR	MS	5
X541	R	S	MR	11

*Races 230, 325, and 264B represent three successive and overlapping stages of rust-race evolution. R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.
Source: Frey et al., 1971.

E68' was released in 1968 (Table 32-2). Two years later the mixture was reconstituted on the basis of changes in the rust population and new isolines that were available (Table 32-3). For example, isoline C237-89II was present as 8 percent of 'Multiline E68' but was dropped from 'Multiline E70.'

Mixtures of Closely Related Lines

Closely related lines may be derived from populations that have one common parent. To facilitate selection of lines genetically different from each other and superior to the common parent, the percentage of germplasm from the common parent in each population generally is kept as low as possible.

To develop a mixture of lines with different genes for pest resistance, a series of populations is developed from parents differing in resistance. The populations may involve single crosses or more complex matings. One or two backcrosses to a recurrent parent also may be used to develop populations in which selection can be practiced.

The procedure used for multiline development in wheat by the International Maize and Wheat Improvement Center (CIMMYT) is based on closely related lines. Rajaram and Dubin (1977) described the development of a multiline at CIMMYT that utilized the semidwarf cultivar 'Siete Cerros' as the common parent in crosses. They indicated that individual components were selected from double-cross populations derived from crosses of 'Siete Cerros' with over 500 cultivars or lines from Argentina, Australia, Canada, Colombia, Ecuador, India, Kenya, North Africa, Rhodesia, the United States, and other areas. The parents were chosen for their diverse origins and their proven resistance to stem rust, leaf rust, and *Septoria* diseases. Pairs of single crosses were mated to form double-cross populations, such as ('Siete Cerros' × parent A) × ('Siete Cerros' × parent B). Segregates in the double-cross populations that were phenotypically different from 'Siete Cerros' were not considered as potential components of the multiline. Rajaram and Dubin indicated that the use of double-cross populations was more rapid and provided more valuable genetic variability among multiline components for genes other than rust resistance than would be possible with the use of backcrossing to obtain isolines.

A mixture of closely related lines was developed and released in India as 'KSML3' (Gill et al., 1980). The six components of the multiline were derived from crosses with the cultivar 'Kalyansona' as the common parent. Several different types of crosses were used to develop each of the components, including single crosses and limited backcrossing.

A program designed to develop closely related lines with limited backcrossing would be initiated in the same manner as one for development of highly related isolines. After one or two backcrosses, lines would be selected for resistance of the donor parent and evaluated for yield and other agronomic characters. Superior lines from the different populations would be used to form the mixture for commercial use.

The potential value of limited backcrossing is that segregates may be obtained from the populations that are superior in yield to the recurrent parent (Borlaug, 1959). When repeated backcrossing is used to develop isolines, the yield of the components is not expected to exceed that of the recurrent parent.

The multilines of peanut that are grown commercially in the southern United States are mixtures of lines with superior quantitative traits and similar phenotypic appearance that are selected from the same breeding population (Norden, 1980). 'Florigant' is composed of seven lines, and 'Florispan,' 'Early Runner,' and 'Dixie Runner' each are mixtures of four lines.

Breeder seed of a multiline of closely related lines is prepared by increasing each component separately, then mixing an equal amount of seed from each by weight. The breeder seed is used as the basis for future generations of multiplication of the mixture for commercial use.

Mixtures of Distinctly Different Cultivars or Lines

Mixtures of distinctly different cultivars can be utilized when phenotypic uniformity is not required. Improvement in the performance of such a mixture occurs as superior lines are used as substitutes for current components. New component lines can be developed by any of the methods that are practical for the species. For example, in self-pollinated species the pedigree, bulk, early-generation testing, and single-seed descent methods are alternatives that could be used to develop superior lines for a multiline. The development of a superior line first involves evaluation of its performance as a pure line. Only lines with superior pure stand performance are evaluated as potential blend components.

EVALUATION OF MIXTURES

Mixtures of isolines, closely related lines, or distinctly different lines are evaluated before release for commercial use. Several factors must be considered in planning alternative mixtures for evaluation.

Number of Components and Their Frequency

The number of components that have been used in mixtures ranges from 2 to 10 or more. The number depends on the purpose of the mixture and the variability and productivity of available lines. Mixtures with two or three components frequently are used to provide a unique product for marketing or to minimize the impact of a deficiency in a superior cultivar. A greater number of lines is preferred when the objective is to provide heterogeneity for pest resistance.

The number of lines in a mixture used for pest control cannot exceed the number of different genotypes for resistance that is available. This number

generally equals the number of donor parents available when isolines are developed. Variability also may depend on the resources available to develop multiple sources of resistance in highly productive lines.

Productivity of the lines available is an important factor in determining the number of components, because yield of the mixture will be close to the weighted mean yield of the components in pure stand (estimated yield). Lines must have a high yield potential in the presence as well as in the absence of the pest. The frequency of each component should be high enough to provide protection against the production hazard being considered.

The performance of a mixture in a species grown commercially for seed can be closely estimated from the weighted yield of the components when grown in pure stand. The yield of each component in pure stand is multiplied by its frequency in the mixture. The sum of the yields computed for each component is the estimated yield of the mixture.

$$\text{Estimated yield} = \Sigma \text{ (yield of component i in pure stand} \times \text{frequency of component i in mixture)}$$

For example, assume that component A yields 100 units and component B yields 110 units in pure stand. The estimated yield of a mixture of 25 percent A and 75 percent B would be $(100 \times 0.25) + (110 \times 0.75) = 107.5$ units.

Deviations from the estimated yield can occur due to competition between the components, commonly referred to as intergenotypic competition. Four types of intergenotypic competition have been defined: undercompensation, complementary compensation, neutral compensation, and overcompensation (Schutz and Brim, 1967). Neutral compensation occurs when the components yield the same in the mixture as they do in pure stand; consequently, the yield of the mixture is equal to the estimated yield. For the three other types of competition, the performance of the components with the better competitive ability increases and that of the poorer competitors decreases. Undercompensation occurs when the increase in performance of the better competitors is less than the decrease in performance of the poor competitors. The yield of a mixture exhibiting undercompensation is less than its estimated yield. Complementary compensation is present when the increase in performance of one or more components is equal to the decrease in performance of the other component or components. A blend with complementary compensation has the same yield as estimated by the performance of the components in pure stand. Overcompensation occurs when the increase in performance of one or more components exceeds the decrease in performance of the other components. The performance of a mixture displaying overcompensation exceeds its estimated yield.

Assume that components A and B are grown together in a 1:1 mixture. The yield of component A in pure stand is 200 units and that of B is 180 units. If the yield of A is 100 units and that of B is 90 units, neutral compensation has occurred, because the yield of each component is equal to one-half its pure stand yield. When A yields 120 and B 60 units, undercompensation has occurred,

because the yield increase in A (20 units) is less than the yield decrease in B (30 units). If A yields 120 and B yields 70 units, complementary compensation has occurred, because the yield increase in A (20 units) is equal to the yield decrease in B (20 units). A yield of 130 units of A and of 80 units of B represents overcompensation, because the yield increase in A (30 units) is greater than the yield decrease in B (10 units).

A survey of data on multiline performance of various crop species indicates that deviations from the estimated yield may occur, but the percentage change is usually less than 3 percent of the estimate when averaged over a number of environments. Several principles for multiline evaluation can be drawn from such information.

1. A superior mixture requires component lines that are superior when grown in pure stand.
2. When it is necessary to mix components that differ in performance, the one with the best performance should be used in the highest frequency possible. This principle is particularly important when a mixture is used to minimize the impact of a defect in a superior cultivar. Assume that component A yields 300 units and B 250 units. In the absence of damage to A, the estimated yield of a blend of 0.25 A and 0.75 B would be 262.5 units, of a blend of 0.50 A and 0.50 B would be 275 units, and of a blend of 0.75 A and 0.25 B would be 287.5 units. The optimum frequency of the components would be one that kept A in the highest proportion without unduly sacrificing the protection provided by B.
3. Identification of a mixture that exhibits overcompensation requires extensive testing over multiple environments. A yield change of only 3 percent requires extensive evaluation to differentiate between a true yield increase and experimental error. A breeder must decide if the resources required to identify a mixture with over compensation might not be better spent evaluating lines that may provide superior performance.

COMMERCIAL SEED PRODUCTION OF MIXTURES

The two steps in production of a seed mixture for commercial plantings are production of pure seed of each component and mixing of seed of the components some time before the mixture is distributed to farmers. These steps can be considered in relation to the classes of certified seed: breeder, foundation, registered, and certified (Chap. 36). The registered and certified classes are the ones available to farmers for planting.

The primary variable in seed production of a mixture is the number of generations of seed multiplication that occurs between the time the components are mixed and the time the mixture is planted by the farmer. The number of generations of seed multiplication ranges from three to zero. Three generations

are used when breeder seed of the components is mixed and used to produce foundation, registered, and certified seed. No generations of seed multiplication are used when seed of the components is mixed immediately before it is distributed to farmers.

The variation in seed production practices is associated with the relative importance of intergenotypic competition in altering the proportions of components during seed multiplication. The frequency of components is altered whenever competition increases the number of seeds produced by a good competitor and decreases the number produced by a poor competitor. For example, a seed mixture of 'Provar' and 'Amsoy 71' soybean in a 1:1 ratio was planted in two Iowa locations (Fehr, 1973). The yield of 'Provar' in the mixture increased an average of 21 percent, while the yield of 'Amsoy 71' decreased 16 percent. If the harvested seed was planted, the proportion of 'Provar' and 'Amsoy 71' would be considerably different from the 1:1 ratio planted the previous generation.

The procedure of producing three generations of seed after mixing the components has been used primarily for mixtures of isolines or closely related lines. One reason for the procedure is that it is difficult to increase a larger number of isolines independently and to mix their seed immediately before distribution to farmers. It is much easier to produce and mix a small quantity of seed of each component and multiply the mixture. Another reason is that intergenotypic competition between isolines is assumed to be absent or of minimal importance. That assumption was brought into question by Murphy and colleagues (1982), who evaluated the composition of an oat mixture of five isolines differing in resistance

Table 32-4 Percentage of Five Isolines in an Oat Mixture During Four Generations of Seed Multiplication in Environments with Rust Absent and Present

		Percentage of Isoline in Designated Generation				
Environment	Isoline	0	1	2	3	4
Rust-free	CI 9192	22	18	16	14	10
	CI 9183	18	23	19	19	14
	CI 9184	20	22	31	26	38
	CI 9190	21	23	21	24	20
	CI 9191	19	14	13	17	18
Rust present	CI 9192	22	19	19	14	15
	CI 9183	18	20	19	17	22
	CI 9184	20	22	25	28	28
	CI 9190	21	23	25	24	19
	CI 9191	19	16	12	17	16

Source: Murphy et al., 1982.

to crown rust. They observed in rust-free environments an increase in the proportion of one component from 20 to 38 percent and a decrease in another component from 22 to 10 percent after four generations of seed multiplication (Table 32-4).

The only way to ensure that the desired proportion of components is present in a mixture is to mix the seed immediately before it is sold to the farmers. That is the procedure used by most seed companies that merchandise mixtures of different cultivars or lines.

Production of the Components

The components of a mixture may be increased in small quantities as breeder seed or in large quantities as certified seed. The purification and multiplication of a component of a self-pollinated species involves the procedures described in Chap. 31 for a pure-line cultivar. When a certified mixture is prepared, each component must pass all field and seed tests of a homogeneous cultivar before the seed is mixed.

Preparation of the Mixture

The most accurate method of preparing a mixture is to mix the components to the desired proportion on the basis of number of viable seeds. Mixtures generally are prepared on the basis of weight; therefore, number of viable seeds is converted to the weight of each component required to achieve the desired proportions. The steps in preparing a mixture are as follows:

1. Determine the germination percentage and number of seeds per unit for each component.
2. Compute the number of viable seeds per unit weight of each component by multiplying the germination percentage by the seeds per unit weight.
3. Determine the number of viable seeds of each component required per 100 seeds of the blend. This is equal to the percentage of each component desired in the mixture.
4. Compute the relative weight of each component required to obtain the desired number of viable seeds in the mixture by dividing the percentage of a component by the number of viable seeds per unit weight.
5. Compute the weight of each component per unit weight of the mixture by dividing the relative weight of each component from step 4 by the sum of the relative weights for all components.

Assume that a mixture will be made from three components. Following the steps just outlined, the mixture is prepared as follows:

1. The germination percentage and number of seeds per kilogram are found to be the following:

Component	Germination (%)	Number of Seeds per Kilogram
A	95	3800
B	87	3700
C	92	3900

2. The number of viable seeds per unit weight is computed.
   Component A $0.95 \times 3800 = 3610$ seeds/kg
   Component B $0.87 \times 3700 = 3219$ seeds/kg
   Component C $0.92 \times 3900 = 3588$ seeds/kg
3. The percentage of each component in the mixture is assumed to be component A, 30 percent; component B, 20 percent; and component C, 50 percent.
4. The relative weight of each component required in the mixture is computed.
   Component A $30/3610 = 0.0083$
   Component B $20/3219 = 0.0062$
   Component C $50/3588 = 0.0139$
5. The weight of each component per kilogram of the mixture is computed.

$$0.0083 + 0.0062 + 0.0139 = 0.0284$$

   Component A $0.0083/0.0284 = 0.29$ kg
   Component B $0.0062/0.0284 = 0.22$ kg
   Component C $0.0139/0.0284 = 0.49$ kg

Mixtures are made commercially by mixing the components before or after the seed is cleaned. The most common procedure is to put uncleaned seed of the components together and pass the mixture over a cleaner to accomplish both cleaning and mixing. The percentage of weight of each component that will be removed by cleaning must be determined and the weight of uncleaned seed adjusted to obtain the desired proportion in the cleaned mixture. This procedure is favored because thorough mixing occurs as the seed passes through the cleaner. Storage facilities are required for unclean seed of the components and clean seed of the mixture.

A second procedure is to mix seed after each component has been cleaned separately. The procedure requires storage facilities for both uncleaned and cleaned seed of each component.

Marketing

A seed mixture sold commercially in the United States is given a brand name. The brand name is a designation assigned by the seed merchandiser and is not legally the same as a cultivar name. Under the Federal Seed Act, seed sold as a mixture must be labeled with the names of the components or with the statement "Variety not stated."

Seed Utilization by the Farmer

One of the concerns commonly expressed by farmers about mixtures of self-pollinated species is whether the seed they harvest can be used to plant a crop the following season. The answer depends on the amount of intergenotypic competition that occurs within the mixture and the importance of changes in frequency of the components. The amount of intergenotypic competition and change in component frequency is difficult to determine unless seed or seedlings of the components have distinguishing characteristics. Soybean seed may differ in hilum color and the seedlings may display differences in hypocotyl color. When such differences occur between components, the frequency of each in a mixture can be determined.

Changes in frequency can have an important effect on performance of the mixture. When a mixture is used to minimize the impact of a defect in a superior cultivar, the resistant component that is lower yielding occurs in the lowest frequency possible. If intergenotypic competition or presence of the production problem favors the resistant component during seed production, the frequency of this component will increase in the harvested seed. The performance of the mixture may decrease the following generation because the proportion of the lower yielding component is unnecessarily high.

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CHAPTER THIRTY-THREE

Development of Synthetic Cultivars

A synthetic cultivar is prepared by intercrossing selected clones or inbred lines. The seed used commercially is obtained by open-pollination. Synthetic cultivars are used for many forage grass and legume species throughout the world. They are not used in the United States for crop species that can be grown commercially from hybrid cultivars, such as maize. In some countries, however, synthetic cultivars of maize and other crops are more widely used than hybrids.

The breeding procedures used to develop a synthetic cultivar depend on the feasibility of developing superior inbred lines or clones. For species such as maize, inbred lines are developed by the same procedures used for development of hybrid cultivars. For many forage species, inbreeding depression is too severe to permit the formation of inbred lines, but the parents can be maintained and reproduced readily by clones. The primary focus of this chapter will be on the latter species.

The factors to consider in the development of a synthetic cultivar include (a) formation of a population, (b) evaluation of individual clones per se, (c) evaluation of the combining ability of a clone, (d) evaluation of experimental synthetics, and (e) preparation of seed for commercial use. Alternative procedures are available to the breeder for most aspects of a cultivar development program.

POPULATION FORMATION

There are several sources of breeding populations from which clones are derived. For a species that is native to a country, natural open-pollinated populations

often are used to initiate breeding programs. The native populations also can be valuable to breeders in countries where the species has been introduced.

Open-pollinated cultivars, indigenous and introduced, are commonly used as breeding populations. Seeds from a livestock pasture or other grazing area represent a population that can be used for selection. Any cultivar, regardless of its year of development, is a potential breeding population.

Populations can be formed by planned crosses among selected clones, both indigenous and introduced. Populations that are being improved by recurrent selection can be a useful source of genetic variability. Each cycle of selection provides a new opportunity for superior clones to be obtained. Improved populations also may be released directly as synthetic cultivars.

IDENTIFICATION OF SUPERIOR CLONES

The methods used to identify superior individuals for use in a synthetic cultivar involve phenotypic selection, genotypic selection, or both. Phenotypic selection is based on individual plant performance or clonal evaluation. Genotypic selection is based on the progeny performance of an individual, including testcross (topcross) and polycross progeny. Genotypic selection also can include selfed progeny for species that can be inbred without excessive loss in vigor.

Phenotypic Selection

Individual Plant Selection. The first step in a breeding program generally is the identification of individual clones with desirable phenotypic appearance. It is common to plant seeds of a population in the greenhouse, evaluate the seedlings for resistance to important diseases and insects, and transplant the seedlings to the field for further evaluation. The field chosen for the planting may be appropriate for further evaluation of pest resistance. The seedlings are planted far enough apart that they remain separated, despite their tendency to spread by vegetative propagules. Seed production on the clones may be prevented if there is concern about contamination from open-pollinated seeds that germinate in the nursery. Individual plant data may be taken for many traits, including regrowth after cutting, forage quality, and winter hardiness.

Selected clones often are transplanted to a maintenance nursery where they are kept during subsequent years of evaluation. The maintenance nursery is a source of propagules for use in a replicated clonal evaluation or for plantings to obtain seed for genotypic evaluation.

Individual plant selection is used for population improvement by recurrent phenotypic selection. The improved populations can be released directly as synthetic cultivars. Alternatively, each clone that is selected for crossing to form a

new population can be evaluated further for possible use in synthetic cultivars developed from a limited number of parents.

Clonal Evaluation. Clonal evaluation is conducted by replicated testing of individuals reproduced by asexual propagation. It is phenotypic rather than genotypic evaluation, because the individual itself is tested, not its offspring. Clonal evaluation may be used to identify phenotypically superior individuals before tests for combining ability are conducted. Hanson and Carnahan (1956) discussed the use of clonal evaluation for the breeding of perennial forage grasses. They indicated that the value of clonal tests depends on the degree of association between the performance of the clones per se and their combining ability when crossed with other clones. They noted reasonably high correlations between performance of a clone and its combining ability for maturity, leaf width, disease resistance, and habit of growth. The correlations were low for leafiness, seed yield, height, vigor, forage yield, and regrowth after cutting. They concluded that clonal evaluation is not a substitute for genotypic evaluation, but does permit the elimination of inferior individuals before expensive replicated tests for combining ability are initiated. They indicated that phenotypically desirable plants from a population can be evaluated in clonal plots in connection with the production of seed for combining ability tests.

Genotypic Selection

Genotypic selection refers to an evaluation of the ability of a clone to produce progeny with superior performance when crossed with other clones. The methods used to evaluate the combining ability of a clone can be divided into two categories: (a) the testcross method, in which the tester is a heterogeneous population, and (b) the polycross method, in which the tester is the clones being evaluated.

Testcross Evaluation. Testcross evaluation, also referred to as a topcross test, is used to determine the general combining ability of an individual. Those individuals that perform well in the testcross evaluation are advanced to trials in which they are evaluated in crosses with other selected individuals.

The principal steps involved in a testcross evaluation are selection of a tester, production of testcross seed, and the testcross trial.

Selection of a Tester. For testcross evaluation, the tester is a heterogeneous population that produces gametes with diverse genotypes. The diversity of gametes permits an assessment of the average ability of a clone to produce superior progeny when combined with genes from many other individuals. An appropriate tester is one that will maximize differences in the performance of the clones

being evaluated. The most common testers for forage grasses and legumes are widely used cultivars or experimental synthetics.

Production of Testcross Seed. A testcross evaluation is of greatest usefulness when the variation in performance among the progeny of clones is due to the genetic potential of the clones themselves and not due to variation in the genetic contribution of genes from the tester. Any variation in the average contribution of genes from the tester is a source of experimental error that will make selection among the clones less reliable. The primary concern, therefore, in the production of testcross seed is that all of the clones should be mated to a similar array of gametes from the tester. Special precautions often must be taken to prevent nonrandom pollination from occurring.

In most forage species, testcross seed is produced on clones that are not male-sterile or that cannot be emasculated readily. Pollen is available from the clone itself (self-pollination), from other clones being evaluated, and from the tester. Self-pollination is not a major concern, because the species generally has some degree of self-incompatibility or because pollen from another genotype is more likely to effect fertilization than its own pollen. The main source of nonrandom pollination is undesirable matings with adjacent clones in the testcross nursery, rather than matings with the tester.

Five factors in the layout of a testcross nursery that are considered to minimize nonrandom pollination include isolation, the direction of the prevailing wind, the proximity of the tester to the clones, the distance between clones, and replication of the clones.

1. *Isolation*: The nursery should be adequately isolated from other plantings of the same species. Isolation distances required for the production of certified seed of the species can be used as a guideline for isolating the testcross nursery. Isolation also can be aided by surrounding the nursery with several rows of the tester to provide an adequate supply of pollen from the desired source (Fig. 33-1).
2. *Direction of the prevailing wind*: For wind-pollinated species, rows of the tester and of the clones should be planted perpendicular to the prevailing wind (Fig. 33-1). This will maximize the movement of pollen from the tester to the clones instead of between clones.
3. *Proximity of the tester to the clones*: The tester should be planted as close as possible to the clones to minimize the distance of pollen transfer. The distance between the tester and clones should be enough, however, to ensure that they do not grow together before the seed is harvested.
4. *Distance between clones*: Pollen transfer between clones can be minimized by increasing the distance between them in the row. The distance will be influenced by the species involved and the amount of land available.
5. *Replication of the clones*: Replication of clones in the testcross nursery will minimize the effect of crossing between clones (Fig. 33-1). In each replication, the clones are planted in a different order. For each clone,

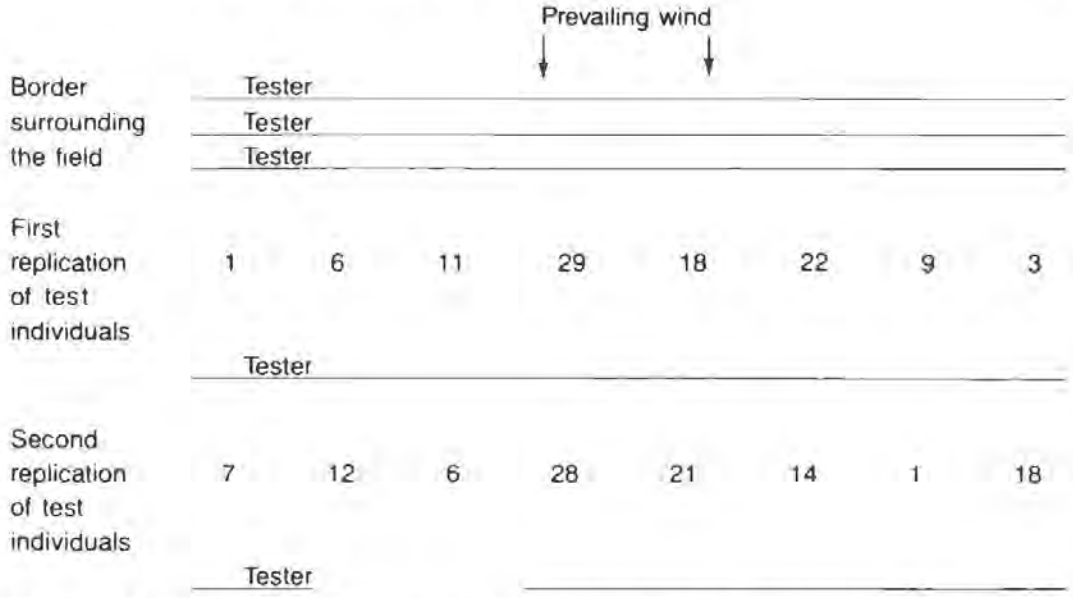


Figure 33-1 Layout of a nursery for production of testcross seed of clones to be evaluated for their combining ability.

seed is harvested from each replication and an equal quantity of seed from each replication is bulked for testing purposes. If seeds were obtained from crossing between clones in one replication, seeds from other replications would decrease their frequency in the testcross seed.

The rows of the tester generally are planted with seed, and the clones are planted with vegetative propagules. For asexually propagated species whose vegetative parts cannot be maintained in storage, each test individual is grown in a separate maintenance nursery. If the clone is found to be superior in the testcross evaluation, propagules from the maintenance nursery are available for further plantings.

Testcross Trial. The testcross seed is used to grow replicated tests for evaluation of characteristics important for the species. Superior clones that are identified on the basis of their testcross performance generally are evaluated further in a polycross test.

Polycross Evaluation. A polycross test is a method of genotypic selection among clones that are being considered for use in a synthetic cultivar. It differs from a testcross in that polycross seed of a clone is obtained by pollination with other selected clones rather than by pollination with an outside tester. Like a testcross, a polycross is intended to evaluate the general combining ability of a clone.

The concept of polycross evaluation was suggested by Frandsen (1940) on the basis of his experience with breeding forage species in Denmark, and by Tysdal and colleagues (1942) on the basis of research in alfalfa breeding. Frandsen was interested in a method of selecting clones that could be used to develop

superior cultivars, a process he referred to as strain-building. Frandsen did not use the term polycross, but the procedure he described became known by that name. Tysdal and co-workers developed the polycross concept through their interest in adapting new maize breeding concepts to alfalfa. Because they did not mention the work of Frandsen in their paper, it is assumed that they developed the concept independently.

The effectiveness of a polycross is determined by the degree to which random pollination occurs among the clones. Random pollination occurs when each clone has an equal opportunity to be pollinated by any of the other clones. When random pollination occurs, the variation in progeny performance among the clones being tested is due primarily to the genetic potential of the clone and not to the genetic contribution of the pollen source.

Factors to be considered in developing an effective polycross nursery are the timing of flowering, isolation, the number of replications, and the arrangement of clones within the replications.

1. Random pollination can only occur if the clones are flowering at the same time. The flowering characteristics of a clone should be evaluated before the polycross is established. Only those with similar time of flowering should be included in the polycross.
2. The polycross nursery should be isolated by an adequate distance from other plants of the same species. The isolation distances required for the production of certified seed of a species can be used as a guideline for isolating the polycross nursery.
3. Replication is essential to obtaining random pollination. Replication is required because adjacent clones are the most likely to intercross. If only one replication is used, the seed from a clone will not represent a random array from the potential pollen source, and the performance of the progeny may not reflect its genetic potential relative to other clones.
4. The two most common designs for arrangement of clones within replications of a polycross nursery are the randomized complete-block and the Latin square. The arrangement of clones in these designs is the same as if they were being used to collect data in a conventional field experiment. Details on the designs for a replicated polycross are discussed in Chap. 12.

The seed from each replication of each clone in a polycross is harvested separately. For each clone, a similar quantity of seed from each replication is bulked. The seed is used to evaluate the combining ability of each clone in a replicated test.

Unreplicated Polycross Nursery. Some breeders use the term polycross to denote the intercrossing of selected clones, regardless of the amount of replication. Others prefer to use the term open-pollinated progeny evaluation in referring to genotypic selection among clones with seed obtained from an unreplicated nurs-

ery. The general procedure is to evaluate unreplicated clones for important characters during one or more seasons. The selected clones are allowed to intercross by open-pollination and the seed from each clone is harvested separately. The seed is used to evaluate the combining ability of each clone in replicated tests.

The primary advantage of the open-pollinated progeny evaluation is the saving in time and labor afforded by obtaining seed for genotypic evaluation without establishment of a special testcross or replicated polycross nursery. The disadvantage arises from the fact that the pollen source for each clone is not the same, because each tends to be pollinated more frequently by the clones most adjacent to it. As a result, variation in the progeny performance of the clones being tested is due to the genetic potential of the clone itself and to the genetic contribution of the pollen source. The variation due to the pollen source is experimental error, which reduces the ability to detect real genetic differences among the clones.

EVALUATION OF EXPERIMENTAL SYNTHETICS

After superior clones have been identified, they may be mated in various combinations to produce experimental synthetics. An experimental synthetic that is superior can be released as a cultivar.

The number of synthetic cultivars that can be developed increases rapidly as the number of potential parents is increased (Table 33-1). It is advantageous to be able to predict the performance of synthetics before their formation. Formulas have been developed for this purpose. Wright (1922) used the formula

$$F_2 = \bar{F}_1 - \frac{(\bar{F}_1 - \bar{P})}{n}$$

to predict the performance of synthetic cultivars in a diploid species. F_2 is the predicted performance of the synthetic, $\bar{F}_1$ is the mean performance of all possible

Table 33-1 Relationship Between Number of Available Syn 0 Parents and Number of Possible Combinations of Parents in Synthetics

Number of Parents	Number of Possible Synthetics
4	11
6	57
8	247
10	1013
12	4083
n	$2^n - n + 1$

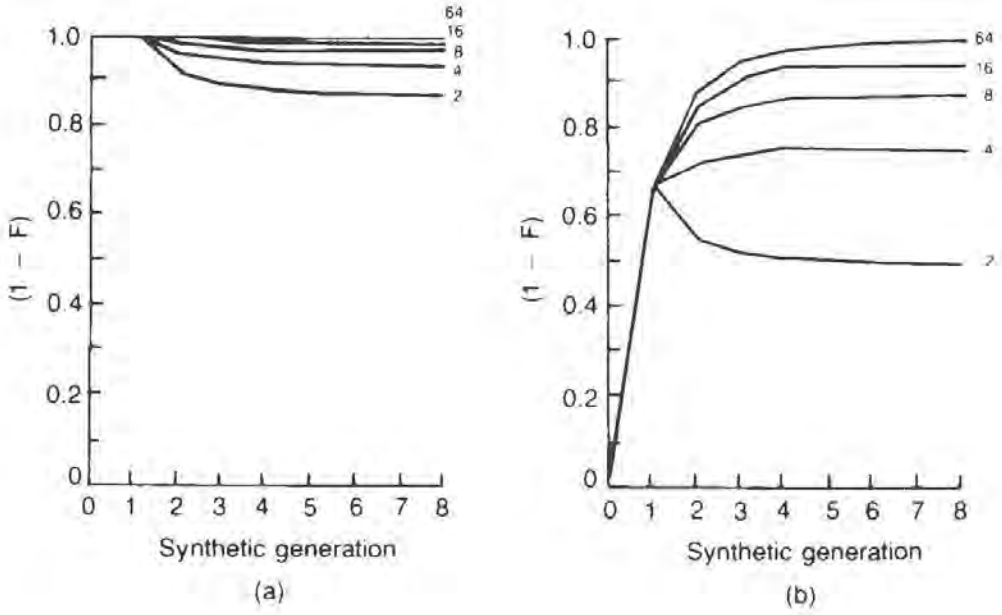
single crosses among n parents, and $\bar{P}$ is the mean performance of n parents. Random mating from the F_1 to F_2 generations and a lack of epistasis are necessary to obtain a good relationship between the predicted and empirical values for the F_2 generation. From the formula it is apparent that the performance of a synthetic can be improved by increasing the combining ability of the parents ($\bar{F}_1$), increasing the number of parents (n), or increasing the performance of the parents ($\bar{P}$). It is difficult to maintain a high value for $\bar{P}$ and $\bar{F}_1$ as n increases.

Busbice and Gurgis (1976) developed prediction formulas that can be used for autopolyploid species. The computations require data on the performance of selfed progeny plus either clonal, polycross, or single-cross progeny. In practical tests of various theoretical formulations, predictions of synthetic performance based on logarithmic formulas of selfed and single-cross progeny performance were found to give the best fit to observed results.

The performance of synthetic cultivars is influenced by the level of inbreeding in the synthetic cultivar. The level of inbreeding is in turn dependent on the number of parents used in forming the synthetic, the degree of inbreeding of and relationship between the parents, the ploidy level of the species, and the frequency of selfing during random-mating generations.

The number of parents in a synthetic may range from 2 to more than 100. The degree of inbreeding in a synthetic cultivar decreases as the number of unrelated parents mated to form the synthetic increases. Busbice and colleagues

Figure 33-2 Expected inbreeding in eight generations of multiplications of synthetic cultivars originating from (a) unrelated noninbred autotetraploid parents and (b) unrelated homozygous autotetraploid parents. Random mating is assumed to be complete and selfing is assumed to be absent. The numbers at the right of each graph indicate the number of parents in the Syn 0. (Courtesy of Busbice et al., 1972.)



(1972) suggested, however, that the use of more than 16 unrelated parents provides little additional advantage (Fig. 33-2). As the number of parents intermated increases, identification of high-performing parents with good combining ability becomes more difficult. In the case of parents that are related or whose relationships are unknown, the use of more than 16 parents may be advisable.

The amount of inbreeding that occurs in a synthetic cultivar affects the generation of seed that is used in the evaluation of an experimental synthetic. The parents of a synthetic are called the Syn 0 generation, and successive random-mated generations are called the Syn *n*, where *n* is the number of generations of random mating that occurred in formation of the synthetic. In autotetraploid species, the evaluation of the Syn 2 generation, rather than of the Syn 1, is recommended (Busbice et al., 1972). The change in the performance of a synthetic due to inbreeding is greatest from the Syn 1 to the Syn 2 generation (Fig. 33-2). The Syn 2 seed used to evaluate an experimental synthetic may approximate its performance in later generations.

SEED PRODUCTION OF A SYNTHETIC CULTIVAR

The parents used to develop the synthetic are intercrossed by hand or are open pollinated to obtain the Syn 1 generation. The ideal is to cross every parent to every other one, obtain a similar number of seed from each mating, and bulk the seed of each mating to form the Syn 1. This ideal may be difficult or impossible to achieve for some species.

A polycross is most commonly used to obtain Syn 1 seed by open-pollination among selected individuals that can be clonally propagated and have a high degree of self-sterility or self-incompatibility. Randomized complete-block or Latin square designs with multiple replications are commonly used to maximize the change of random pollination. The factors discussed in Chap. 12 with regard to formation of a population by a polycross apply equally well to the production of Syn 1 seed of an experimental or commercial synthetic.

Seed of the Syn 2 generation is produced by open-pollination of plants established from Syn 1 seed. When a large area of production is involved, the planting and harvest are carried out with equipment used by farmers for conventional agricultural production.

The Syn 3 generation is produced by open-pollination of plants established with Syn 2 seed. In the same manner, the Syn 4 is produced from the Syn 3.

SEED USED FOR COMMERCIAL PRODUCTION

The classes of seed for synthetic cultivars used in the United States are breeder, foundation, and certified. The same classes of seed are available internationally, although they may be designated by different terms.

Breeder Seed

Breeder seed represents the source from which all other classes of seed are developed for commercial use. The generation involved generally is the Syn 1; however, the Syn 2 generation is employed.

The maintenance of adequate quantities of breeder seed is accomplished by regular resynthesis of the synthetic or by maintaining adequate quantities of the initial breeder seed in storage for the life of the cultivar. The latter procedure is common for perennial species, because foundation seed can be produced for several years from stands established with breeder seed. It is possible to retain enough breeder seed in storage for periodic reestablishment of a planting to obtain foundation seed.

Breeder seed of a perennial species may be harvested from a field of the cultivar for more than 1 year. For example, in alfalfa it is common to harvest breeder seed for 2 years from a field. The number of years of production is restricted, because plants that develop from shattered seed before or during harvest can cause contamination of the original stand.

Foundation Seed

The generation of open-pollinated seed harvested from a stand established with breeder seed generally is referred to as foundation seed. In some cases, the foundation class is omitted and the seed is sold as the certified class for commercial plantings. The Syn generation of foundation seed is one greater than that of breeder seed.

For perennial species, there is a limited number of years that a stand of breeder seed can be used to produce foundation seed. In alfalfa, the limitation is generally 3 years but has been as high as 5 years. In some cases, foundation seed put in storage is used throughout the life of the cultivar to establish stands from which certified seed can be harvested.

Certified Seed

The open-pollinated seed harvested from a stand established with foundation seed is referred to as certified seed. Certified seed is the class most commonly used for commercial planting. Seed sold for commercial planting from a stand established with breeder seed is commonly classed as certified seed, instead of foundation. In some cases, certified seed is used to establish a stand from which additional certified seed is produced. The seed is one Syn generation advanced beyond that of the seed from which it was produced.

The number of years that certified seed can be harvested from a stand of a cultivar is restricted. In alfalfa, the limitation is 5 years of seed production from a stand established with foundation seed.

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CHAPTER THIRTY-FOUR

Development of Hybrid Cultivars

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

FORMATION OF A SEGREGATING POPULATION

Natural Populations or Open-Pollinated Cultivars

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

Gamete Selection

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

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

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

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

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

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

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

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

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

Crosses between Inbred Lines

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

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

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

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

Populations Derived by Recurrent Selection

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

INBREEDING OF THE POPULATION

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

Amount of Inbreeding

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

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

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

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

Methods of Inbreeding

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

hampered by atypical performance of genotypes. The evaluation phase of early-generation testing could not be done in the off-season nursery, but the nursery could be used to produce the testcross seed necessary for the replicated trial. The single-seed descent method could be used effectively in the off-season nursery to permit rapid inbred line development. A combination of methods often provides an appropriate inbreeding strategy when certain environments can be used only for inbreeding.

EVALUATION OF COMBINING ABILITY

General Combining Ability

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

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

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

The testers most commonly used today for the first evaluation of combining

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

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

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

Specific Combining Ability

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

Prediction of Three-Way and Double-Cross Hybrid Performance

The evaluation of a group of inbred lines for the production of a three-way or double-cross hybrid is hampered by the number of cross-combinations that are

possible. The formulas for determining the number of possible crosses among a group of n parents, excluding reciprocals, are as follows:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

SPECIAL CONSIDERATIONS WITH CYTOPLASMIC-GENETIC MALE STERILITY

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

Male Parents

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

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

Female Parents

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

The breeder begins with a segregating population of male-fertile plants that have normal cytoplasm and nonrestorer alleles. The population must be inbred, the combining ability of genotypes must be determined, and B line and A line versions of a superior inbred must be developed. In crops such as maize in which hybrid seed can be readily produced by artificial hybridization, a population can

be inbred by self-pollination and the combining ability of male-fertile B lines can be determined by manually crossing them to R line testers. When a B line is found that would be useful in a commercial hybrid, the A line version can be developed by backcrossing. This option is not practical, however, for crops in which the quantities of testcross seed needed to evaluate combining ability cannot be produced artificially, such as sorghum.

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

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

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

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

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

Season 4+: (a) The cms-BC_1F_1 and F_5 progeny of each selected F_3 plant are grown in adjacent rows. Three F_5 plants in each selected row are crossed to the adjacent cms-BC_1F_1 to obtain BC_2F_1 seed. The selfed F_6 seed and BC_2F_1 seed for each F_5 plant are harvested to continue inbreeding, selection, and backcrossing in season 5. (b) Two cms-BC_1F_1 plants are crossed to an R line tester different from the one used in season 3. The testcross seed is used to evaluate the line for general combining

ability in season 5. (c) Five BC_1F_1 plants are bagged to evaluate self-fertility. (d) The cms-F_1 × R line testcrosses are evaluated for yield and other important traits in replicated tests. Lines that perform poorly are discarded, including backcross and inbred seed prepared in season 4.

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

IMPROVEMENT OF INBRED LINES BY BACKCROSSING

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

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

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

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

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

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

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

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

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

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

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

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

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

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

PREPARATION OF BREEDER SEED

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

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CHAPTER THIRTY-FIVE

Hybrid Seed Production

Hybrid seed is used for the commercial production of a number of crops. Each of these crops must meet four requirements for the successful production and use of hybrid seed. (a) Heterosis is exhibited by the F_1 progeny of crosses between the parents. (b) Fertile pollen can be eliminated from the female parent. (c) Pollen from the male parent is effectively transported to the female parent. (d) Hybrid seed can be produced reliably and economically.

REQUIREMENTS FOR HYBRID SEED PRODUCTION

Heterosis

Heterosis (hybrid vigor) is present when the performance of the F_1 progeny of a cross exceeds that of the parents. The F_1 progeny can be compared with the mean performance of the parents, referred to as mid-parent heterosis, or to the performance of the best parent, referred to as high-parent heterosis (Chap. 9). To justify the use of hybrid seed, high-parent heterosis must be present.

The production of hybrid seed is more expensive than the multiplication of pure-line or open-pollinated cultivars. The performance of the hybrid must sufficiently exceed the performance of other types of cultivars available for the species to offset the added cost of hybrid seed production. For example, sunflower can be produced commercially as open-pollinated cultivars or as hybrids. Hybrids are used in the United States because there is adequate high-parent heterosis present and the hybrids yield more than open-pollinated cultivars.

Elimination of Fertile Pollen from the Female Parent

The two methods for eliminating fertile pollen from the female parent are artificial emasculation and use of male sterility. Artificial emasculation of male-fertile females can be accomplished by manually removing the anthers or by special treatment of the anthers. Manual removal of the anthers is done on a commercial scale for a few crops, such as maize. Chemical or physical treatment of the anthers to make them male-sterile has been tried experimentally, but is not used currently for commercial hybrid seed production.

The types of male sterility available in crop plants are genetic and cytoplasmic-genetic. Systems for utilizing genetic male sterility have been proposed, but none has been adopted at present. The proposed systems are discussed in Chap. 5. Only cytoplasmic-genetic sterility currently is used to produce hybrid seed commercially.

Transfer of Pollen from the Male to the Female Parent

Large-scale production of hybrid seed requires effective pollen transfer from the male to the female parent by wind or insects. Manual pollination is feasible only for crops, such as tomato and petunia, in which performance or demand for the hybrid justifies the cost of the labor required to produce it.

Wind is an effective mode of pollen transport in grass species, such as maize and sorghum. Insect pollination is effective for producing hybrid seed of sunflower if special precautions are taken to ensure that adequate numbers of the appropriate pollen vector are available. Insect pollination of alfalfa has had only limited success in hybrid seed production, because male-fertile plants are preferred by the insects more than male-sterile plants of the female parent. The insect preference results in limited seed set on the male-sterile plants.

Reliable and Economical Hybrid Seed Production

Hybrid seed is feasible for commercial production of a crop when it can be produced reliably and at a cost consistent with performance of the hybrid. The cost of production is related to the amount of quality seed obtained per hectare from the female parent and the amount of seed required to plant each hectare of commercial production.

Yield and quality of seed from the female parent are important considerations in hybrid seed production. In the early years of hybrid maize, yield of the inbred lines was too low to permit reliable and economical production of single-cross hybrids. Double-cross or three-way-cross hybrids were used because the female parent for commercial hybrid seed production was a productive single cross. Today, single-cross maize hybrids are used commercially because breeders have improved the yield of inbred lines sufficiently to permit their use as female parents for commercial hybrid seed production.

Male parents must provide an adequate supply of fertile pollen to pollinate the female parent. Pollen production is a consideration in the development of inbred lines to be used as male parents in hybrid combinations.

The amount of hybrid seed produced per hectare and the amount of seed used per hectare of commercial production are major factors in determining the economic feasibility of hybrid seed. The principle can be illustrated with the following ratio: Number of units of hybrid seed produced per hectare:number of units of seed required to plant a hectare of commercial production.

The economic feasibility for the commercial use of hybrid seed is greatest when the ratio is the largest. For example, the ratio for soybean would be about 20:1, compared with 240:1 for maize. The ratio for soybean assumes that the ratio of the female-to-male rows in a hybrid seed field would be about 1:1, the number of units of hybrid seed per hectare of the female rows would be 40, and that two units of seed would be required to plant 1 hectare of commercial production. For maize, 1 hectare produces about 120 units of hybrid seed and only 0.5 of a unit is needed to plant a hectare. This means that the cost of producing 1 hectare of hybrid soybean seed must be divided among only 20 hectares, but the cost of producing a hectare of hybrid maize would be divided among 240 hectares. It would be much more difficult to make hybrid soybeans economically feasible than it has been for maize.

TYPES OF HYBRID SEED

The type of hybrid produced is a function of the number of parents involved and the relationship among the parents. Some types of hybrids are used in many species, while others are only used in a few. In the following discussion of types of hybrids, the letter P followed by a number (P1, P2, P3, and P4) will be used to designate parents. When two parents in a cross are closely related, commonly referred to as sister lines, one of them will be designated with a P and a number, and the other will have the same designation with an asterisk added to it (P1*).

The degree of relationship between sister lines is not well defined. They may be closely related when P1* is derived from a backcross program in which P1 was the recurrent parent. P1* and P1 may not be as closely related when they trace to different S_0 plants from the same breeding population. Sister lines may result from two or more plant selections from within a line derived in the S_0, S_1, S_2, or a later generation of inbreeding.

Single Cross

A single-cross hybrid is produced by mating two parents, P1 × P2. When cytoplasmic-genetic male sterility is used, the essential aspects of the cytoplasmic and genetic makeup of the parents are

$$\text{P1}(S\textit{rfrf}) \times \text{P2}(\textit{RfRf}) \rightarrow \text{hybrid}(S\textit{Rfrf})$$

S designates the cytoplasm that results in male sterility of the parent when the recessive nonrestorer (*rfrf*) alleles are present in the nucleus. Plants with S cytoplasm are male-fertile when a dominant restorer allele (*Rf*) is present in the nucleus. Plants with normal (N) cytoplasm are male-fertile, regardless if the nuclear alleles are *rfrf* or *Rf*__. In the single cross above, P1 is male-sterile, while P2 and the hybrid are male-fertile. The cytoplasm of the parent with the dominant restorer alleles can be either S or N.

Seed production of a single-cross hybrid and its parents by manual or chemical emasculation requires three separate and isolated plantings: P1 alone, P2 alone, and the P1 × P2 cross. Four separate plantings are required when male stertility is used: A line × B line cross to obtain seed of P1, B line of P1 alone, R line (P2) alone, and the P1 × P2 cross. The cytoplasmic and genetic makeup for the A line of a parent is S*rfrf*, for the B line is N*rfrf*, and for the R line is *RfRf*.

Modified Single Cross

A modified single-cross hybrid is obtained from the cross (P1* × P1) × P2. The P1* × P1 cross provides a female parent that produces more seed than either P1* or P1 alone. The production of a modified single-cross hybrid and its parents by emasculation requires five separate plantings: P1* alone, P1 alone, P2 alone, the P1* × P1 cross, and the (P1* × P1) × P2 cross.

For the production of a modified single-cross hybrid by the use of male sterility, the cytoplasmic and genetic makeup of the parents and the hybrid is

$$[\text{P1*}(\text{S}rfrf) \times \text{P1}(\text{N}rfrf)] \times \text{P2}(RfRf) \rightarrow \text{hybrid}(\text{S}Rfrf)$$

P1* is male-sterile and P1, P2, and the hybrid are male-fertile. Six separate plantings are needed for production of seed of the parents and the hybrid: A line × B line cross to obtain P1*, B line of P1* alone, P1 alone, P2 alone, the P1* × P1 cross, and the (P1* × P1) × P2 cross.

Double Modified Single Cross

The mating used to produce a double modified single-cross hybrid is (P1* × P1) × (P2* × P2). P1* and P1 are closely related lines, as are P2* and P2. The P1* × P1 cross used as a female produces more seed than does either P1* or P1 independently. More pollen production is obtained from the P2* × P2 cross than from either P2* or P2 alone. There are seven separate plantings for the production of the hybrid and its parents by emasculation: P1* alone, P1 alone, P2* alone, P2 alone, and the P1* × P1 cross, the P2* × P2 cross, and the (P1* × P1) × (P2* × P2) cross. The procedure for producing the double modified single cross with cytoplasmic-genetic male sterility is the same as that described for the double cross.

Three-Way Cross

A three-way-cross hybrid is obtained from the mating (P1 × P2) × P3. The female parent is a single-cross hybrid and the male is a third parent. Seed production of the hybrid and its parents by emasculation involves five plantings: P1 alone, P2 alone, P3 alone, the P1 × P2 cross, and the (P1 × P2) × P3 cross.

The cytoplasmic and genetic constitution of the parents and the hybrid with the use of male sterility is

$$[\text{P1}(S\textit{rfrf}) \times \text{P2}(N\textit{rfrf})] \times \text{P3}(\textit{RfRf}) \rightarrow \text{hybrid}(S\textit{Rfrf})$$

P1 is male-sterile and P2, P3, and the hybrid are male-fertile. Production of seed for the parents and hybrid requires six separate plantings: A line × B line cross to obtain P1, B line of P1 alone, P2 alone, P3 alone, the P1 × P2 cross, and the (P1 × P2) × P3 cross.

Modified Three-Way Cross

A modified three-way hybrid is produced from the cross (P1 × P2) × (P3* × P3). The female parent is a single-cross hybrid and the male is the cross between two closely related lines. When emasculation is used, seven separate plantings are required to obtain seed of the parents and the hybrid: P1 alone, P2 alone, P3* alone, P3 alone, the P1 × P2 cross, the P3* × P3 cross, and the (P1 × P2) × (P3* × P3) cross.

For production of a modified three-way-cross hybrid by the use of male sterility, the cytoplasmic and genetic makeup of the parents is

$$[\text{P1}(S\textit{rfrf}) \times \text{P2}(N\textit{rfrf})] \times [\text{P3*}(S\textit{rfrf}) \times \text{P3}(\textit{RfRf})] \rightarrow \text{hybrid: } 1/2\ S\textit{Rfrf}(\text{male-fertile}) \text{ and } 1/2S\textit{rfrf}(\text{male-sterile})$$

P1 and P3* are male-sterile and P2 and P3 are male-fertile. Half of the hybrid plants are male-sterile and half are male-fertile. Nine separate plantings are required to produce seed of the parents and the hybrid: A line × B line cross to obtain P1, B line of P1 alone, P2 alone, A line × B line cross to obtain P3*, B line of P3* alone, P3 alone, the P1 × P2 cross, the P3* × P3 cross, and the (P1 × P2) × (P3* × P3) cross.

Double Cross

The mating (P1 × P2) × (P3 × P4) produces a double-cross hybrid. The female and male parents are single crosses, which provide greater seed and pollen production in the hybrid seed field than do inbred parents. Seven plantings are required to produce seed of the parents and the hybrid by emasculation: P1 alone,

P2 alone, P3 alone, P4 alone, the P1 × P2 cross, the P3 × P4 cross, and the (P1 × P2) × (P3 × P4) mating.

When male sterility is used to produce the hybrid, the cytoplasmic and genetic makeup of the parents and the hybrid is

[P1(S*rfrf*) × P2(N*rfrf*)] × [P3(S*rfrf*)
× P4(*RfRf*)] → hybrid: 1/2 S*RfrfI*(male-fertile) and 1/2 S*rfrf*(male-sterile)

P1 and P3 are male-sterile and P2 and P4 are male-fertile. The hybrid contains male-fertile and male-sterile individuals in a 1:1 ratio. Production of the hybrid and its parents involves nine separate plantings: A line × B line cross to obtain P1, B line of P1 alone, P2 alone, A line × B line cross to obtain P3, B line of P3 alone, P4 alone, the P1 × P2 cross, the P3 × P4 cross, and the (P1 × P2) × (P3 × P4) cross.

Advantages and Disadvantages of the Hybrid Types

Types of hybrids can be compared by considering their rankings with respect to productivity and uniformity in a commercial field, the cost of hybrid seed production, and the number of different plantings required for hybrid seed production.

Productivity. The rank of the hybrid types from the most to the least productive in a commercial field is single cross, modified single cross, double modified single cross, three-way cross, modified three-way cross, and the double cross. The greater productivity of the single cross makes it the preferred hybrid type and the one most widely used for crops in the United States.

Uniformity. The uniformity of plants in a commercial field may be an advantage in obtaining uniform seeds or fruits for marketing and in facilitating harvesting. A single-cross hybrid produced from two inbred parents is the most uniform, because all of the plants are genetically alike. A double-cross hybrid involves genetic segregation of the alleles from four parents and is, on the average, the least uniform of the hybrid types. The rank order of hybrids from the most to the least uniform is single cross, modified single cross, double modified single cross, three-way cross, modified three-way cross, and double cross.

Cost of Hybrid Seed Production. The cost of large-scale production of a hybrid is related to the relative quantity and quality of seed obtained from different types of female parents and the quantity of pollen provided by different types of male parents. Inbred parents have the smallest production of seed and pollen. parents that are the cross between related parents are more productive, and single-cross parents are the most productive. As a result, the single-cross hybrid is the most expensive to produce because both parents are inbreds, and the double-

Table 35-1 Number of Separate Plantings Required to Produce Different Hybrid Types and Their Parents

Hybrid Type	Manual or Chemical Emasculation	Cytoplasmic-Genetic Male Sterility
Single	3	4
Modified single	5	6
Three-way	5	6
Double modified single	7	9
Modified three-way	7	9
Double	7	9

cross hybrid is the least expensive because both parents are single crosses. The rank of hybrid types from the most to the least expensive to produce is single cross, modified single cross, double modified single cross, three-way cross, modified three-way cross, and double cross.

Number of Different Plantings. The production of seed of each parent and of the hybrid itself requires separate plantings that are properly isolated to maintain genetic purity (Table 35-1). The number of different plantings needed depends on the hybrid type that is produced and the use of manual or chemical emasculation or of cytoplasmic-genetic male sterility. The complexity of producing a hybrid increases as the number of separate plantings increases. Separate operations must be conducted for roguing, harvesting, drying, storage, cleaning, inventory, and distribution of each planting.

PRODUCTION OF PARENT SEED

The production of adequate quantities of parent seed is an important part of hybrid seed production. The difficulty of the task increases with the number of lines in the hybrid, particularly when cytoplasmic-genetic male sterility is involved.

The primary objective in producing the parents is to obtain high-quality seed with a high level of genetic purity. Maintenance of genetic purity in species with a high degree of cross-pollination involves elimination of undesired pollen by hand pollination or by isolated plantings. Removal of variation in a parent due to mutation or relic heterozygosity also is a consideration.

Production of seed of an inbred parent has two phases, maintenance of small quantities of pure seed and preparation of larger quantities of seed for use in hybrid seed fields. In many public and private organizations, the breeder is responsible for maintaining pure seed and a separate department handles the preparation of large quantities of parent seed.

Obtaining and Maintaining Pure Seed of an Inbred Line

Pure seed of an inbred line can be obtained by self-pollination or by sib mating.

Self-pollination with progeny testing: The procedure that has the best probability of providing pure seed involves self-pollination and progeny testing. Its primary disadvantages are that repeated generations of self-pollination may reduce the vigor of the line to an undesirable level, and the cost and time required may be greater than for sib mating. The procedure is as follows:

Season 1: Plants within a new inbred line are self-pollinated. Those plants with uniform characteristics are harvested individually, the seed of each is examined, and the ones with similar appearance are retained.

Season 2: A progeny row is grown for each plant retained in season 1. Plants are self-pollinated within each row, and the rows with uniform characteristics are retained. Self-pollinated plants are harvested individually, and their seed is examined for uniform appearance. Part of the seed from each selected plant is saved for progeny testing in season 3, and a similar quantity from each plant is bulked to form the source of pure seed of the inbred.

Season 3: In season 3 and subsequent seasons, pure seed is produced by progeny testing in the manner described for season 2.

Sib mating: The use of plant-to-plant crosses to maintain an inbred line has the advantage of maintaining a higher level of vigor and productivity and of being less expensive than self-pollination and progeny testing. The disadvantage is that the level of genetic purity may be less because selection is based on individual plants rather than on a progeny test.

Season 1: Plants are selected for uniformity before pollination. Selected plants are mated to each other by hand or in isolation. At harvest, plants with uniform characteristics are retained and their seed is bulked. Part of the seed is used for further increase of the inbred, and part is used for continued maintenance of the inbred.

Season 2: The procedure used in season 1 is repeated in season 2 and all subsequent generations to produce breeder seed of the inbred.

Self-pollination and sib mating may be used in alternation to combine the advantages of both procedures. Regardless of the system used, a sample of pure seed first prepared for the parent should be put in storage to retain its viability. The seed can be used periodically as a pure source of the parent free from any genetic change that could occur through multiple generations of self-pollination, sib mating, or both.

Producing Large Quantities of a Parent

Generally it is not practical to produce large quantities of a parent by any form of hand pollination. The parents are planted in isolation, and the seed is produced by open-pollination. Off-type plants are rogued from the fields. Special care is taken to avoid mixtures with other seed lots during harvesting, transport, cleaning, and bagging (Wright, 1980).

Producing Seed of a Male-Sterile Parent

Large quantities of seed of an A line are produced in isolation. The A line (female) is grown in alternating rows with the B line (male) and the seed is obtained by open-pollination. Seed harvested from the A line is used for hybrid seed production, and seed harvested from the B line is used for further production of A line seed.

The male-fertile parents in a hybrid, either N *rfrf* or *RfRf*, are produced in large quantities by growing each in isolation and allowing open-pollination to occur.

PRODUCTION OF HYBRID SEED

Hybrid seed production requires special care to maintain genetic purity and obtain large quantities of high-quality seed. The general principles involved in hybrid seed production were reviewed by Wright (1980).

Field Selection

A field in which seed is produced should be properly isolated from sources of contaminating pollen, including other cultivars and related weed species. The cultural practices used to produce the crop should be those consistent with obtaining high yields, including proper fertilization, weed control, and pest management.

Optimizing Seed Set

Obtaining maximum seed set requires management of the parents to ensure simultaneous flowering and adequate pollen shed. Adequate numbers of the appropriate pollen vector must be provided for insect-pollinated species.

Roguing

Off-type plants in the female and male parents must be removed before they shed pollen.

Artificial Emasculation

Production of hybrid seed for commercial use by artificial emasculation is used only for maize and some high-value horticultural crops. The female parent must be emasculated before pollen shed occurs.

Harvesting

Production of high-quality seed requires harvest at the proper moisture level with suitable equipment. All equipment and storage facilities must be properly cleaned to avoid mixing of seed. Gentle handling of the seed is required to prevent seed damage.

Seed Drying

Some crops are harvested at moisture levels higher than are suitable for seed storage. The seed must be dried at the proper temperature as soon as possible after harvest to prevent loss of germination and vigor.

Seed Conditioning

The steps required to prepare hybrid seed for marketing vary among species. The steps may include cleaning, sizing, treatment of the seed with a fungicide, insecticide, or inoculant, and bagging.

Quality Control

Maintaining genetic purity of hybrid seed involves field and seed inspections. Samples of hybrid seed may be grown in an off-season nursery to determine the frequency of off-type plants. The germination percentage for hybrid seed is always determined before it is sold. Determinations also are made on the amount of weed seed and foreign material.

Storage

Hybrid seed should be stored in facilities that maintain high germination. Temperature and humidity control are important when seed is to be stored for more than 1 year.

Marketing

The merchandising of hybrid seed generally is carried out by a specialized department of a company or organization. Farmers are informed about the available seed through an array of promotional activities.

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CHAPTER THIRTY-SIX

Release and Distribution of Cultivars

The plant breeder has the responsibility to develop and identify cultivars that will enhance commercial production of a crop. The decision to make a cultivar available for widespread use and its actual multiplication and distribution to commercial growers involve many additional persons and agencies. The procedures for release and distribution vary with the type of cultivar, the institution at which it was developed, the procedure for its multiplication and distribution, and the agencies that regulate the distribution process. There also are important differences among countries in the laws that control the release and distribution process. The emphasis in this chapter will be on the procedures commonly used in the United States. To simplify the discussion, the term cultivar will apply to genotypes used for commercial production of a crop or to inbred lines used to produce a commercial hybrid. The term originator will refer to the public institution or private company that developed the cultivar.

THE DECISION-MAKING PROCESS

The decision to release a new cultivar is a stepwise process that usually involves a number of persons, all of whom are responsible for determining if the genotype will be useful for commercial production of a crop. Sufficient performance data must be obtained to convince the breeder and other knowledgeable persons that the genotype merits release. The process is most orderly and efficient when the persons involved have a well-defined procedure to follow. Although exact procedures vary among companies and public institutions, certain principles are common to all.

Role of the Breeder

The breeder is responsible for obtaining the data needed to evaluate the potential of a genotype for commercial use (App. C). It is important that the breeder understand what data are expected by the individuals who ultimately will decide if a genotype will be released. Without that knowledge, the breeder may conduct an inadequate testing program that will prevent the prompt release of a cultivar. In breeding programs of State Agricultural Experiment Stations and the U.S. Department of Agriculture, it may be desirable to release a new cultivar simultaneously in several states. Breeders working in such programs should be aware of the testing requirements of all states that may be interested in release of the cultivar.

The breeder is responsible for coordinating the evaluation of a cultivar with the preparation of breeder seed. Distribution of the cultivar will be most rapid when an adequate supply of breeder seed is available as soon as adequate performance data have been collected. A breeder must work closely with persons responsible for seed multiplication and distribution to ensure that adequate quantities of pure seed are available at the appropriate time.

Role of Persons Other than the Breeder

The final decision to release a cultivar generally is not made by the breeder who developed it (App. C). The persons who make the decision must consider the cultivar's performance relative to that of cultivars currently available and the likelihood that the new cultivar will be readily marketed. As a result, these persons include breeders other than the one who developed the cultivar, agronomists who work closely with farmers, and persons responsible for multiplying and marketing the cultivars. In a private company, an important consideration is the amount of profit that will be realized by use of the new cultivar compared with that obtained with current cultivars.

Although the breeder may not have a vote when the final decision is made, his or her understanding of the factors that are important for release of a new cultivar can directly influence the outcome. Those factors should play a key role in determination of the objectives of a breeding program, development of appropriate sources of genetic variability, and selection and testing of potential cultivars.

Examples of Release Procedures

The principles of the decision-making process that are involved in the release of a genotype will be illustrated by outlining the procedures used by a public

and by a private company. Release of a soybean cultivar will be used in the example for both institutions.

1. Public institution
   The procedures for release and distribution of cultivars by the Iowa Agriculture and Home Economics Experiment Station at Iowa State University are presented in App. C. The procedure described for soybeans will differ in certain details from other crop species, but the principles of the decision-making process will be similar.
   a. First year
      The first replicated test of a line is conducted by the breeder at two locations in Iowa. The lines with the best performance are selected by the breeder without any outside approval.
   b. Second year
      The second replicated test is conducted by the breeder at three locations in Iowa. The breeder selects the best lines without any outside approval.
   c. Third year
      Lines are evaluated in a regional test conducted cooperatively by public breeders in states where the maturity of the line is appropriate. Each breeder who conducts the test recommends whether a line should be discarded or evaluated another year. The final decision on additional testing of lines developed in Iowa is made by the breeder without approval from the Experiment Station.
   d. Fourth year
      Lines are evaluated extensively in the cooperative regional test by all states that are likely to be interested in growing cultivars of that maturity on a commercial scale. All breeders who conduct the test recommend whether lines should be discarded or evaluated again. The final decision for Iowa lines is made by the breeder without approval from the Experiment Station.
   e. Fifth year
      A third year of evaluation in the cooperative regional test is conducted before a line is considered for release. The breeder of a line in Iowa decides if the line warrants consideration for release in that state. If so, a formal request for release must be made to the Agricultural Experiment Station. The Experiment Station requires that a form be prepared by the breeder that documents the attributes of the genotype and the reason for its proposed release (App. D). The request must be approved first by the executive officer of the department to which the breeder is responsible. The executive officer asks the advice and approval of a committee of staff members from the department. The committee consists of plant breeders, extension personnel, and seed production specialists. If the committee approves the request and the

executive officer concurs, the document is signed and forwarded to the Experiment Station.

The associate director of the Experiment Station appoints a review committee of staff from several departments that usually include genetics, agronomy, horticulture, and plant pathology. The breeder who developed the line is not a member of the committee. If approval is given by the review committee and the associate director concurs, the line is released as a cultivar in Iowa. Agricultural Experiment Stations from other states and the USDA are given the opportunity to participate in the release of the cultivar.

2. Private company
   a. First year
      The initial evaluation of soybean lines is conducted in replicated tests at two locations by the breeder. Selection of lines for additional evaluation is done by the breeder without approval from other persons in the company.
   b. Second year
      Lines are evaluated in replicated tests at about five locations. On the basis of the results, the breeder selects lines for additional testing. A form is prepared for each selected line that includes its parentage, morphological characteristics, agronomic performance, and reactions to relevant diseases, nematodes, insects, and soil problems. The breeder decides which lines will be tested the third year.
   c. Third year
      Lines are evaluated at 10 to 15 locations by the breeder who developed them and by breeders at other stations of the company. The breeder uses results of the tests in the third year to select lines that merit additional evaluations and to prepare a detailed description of the performance of each selected line. The decision to test a line the following year must be approved and an appropriate form signed by two other soybean breeders and by the research manager for soybeans. Approval also is required from the manager of the division responsible for production and marketing of a new soybean cultivar.
   d. Fourth year
      A line is extensively evaluated in replicated tests by soybean breeders of the company. It also is evaluated by agronomists of the company in management trials, including row spacing and plant population tests.

      The decision to release a cultivar generally is made after the fourth year of evaluation. The release is based on performance data obtained by the breeders and agronomists. Consideration is given to characteristics important for the production of high-quality seed for sale to farmers. Sales are projected for several years and seed supplies are evaluated. The decision to release a new cultivar must be approved

by the originating breeder, the research manager for soybeans, the person responsible for producing seed of the cultivar, and the manager of the division responsible for production and marketing. Final approval must be given by the president of the company.

DISTRIBUTION PROCEDURES

The distribution of a cultivar to the commercial growers of a crop is a major undertaking. Seed or vegetative propagules must be carefully multiplied and effectively merchandised. The distribution process involves many persons, each with important responsibilities.

Private Companies

Distribution of a cultivar is controlled by the originator or its designated representative. Some private breeding companies maintain direct control over all aspects of multiplication and merchandising. Other companies specialize in plant breeding and obtain a royalty from firms that multiply and merchandise their cultivars. There are companies that specialize in the production and distribution of seed or vegetative propagules to other companies, which in turn make the final multiplication for sale to commercial growers.

Public Institutions

Public institutions that conduct breeding programs include primarily the U.S. Department of Agriculture (USDA) and State Agricultural Experiment Stations. Guidelines for the release and multiplication of cultivars developed by those institutions are described in App. B. Despite these guidelines, there is considerable variation among crops and public institutions in the procedures used to distribute a new cultivar.

A public institution generally is not involved in the direct sale of a cultivar to a commercial grower. The most common strategy is to distribute seed stock of a cultivar to one or more private companies that are responsible for its multiplication and marketing. The originator may permit unrestricted use of the genotype or may regulate its distribution to varying degrees. Some institutions make a cultivar available without charge, and others assess a royalty for its use. This diversity of strategies can be examined by considering independently the philosophies and procedures of the USDA and that of the State Agricultural Experiment Stations.

USDA. A breeder of the Agricultural Research Service (ARS) of the USDA can develop cultivars alone or in cooperation with scientists from State Agricultural Experiment Stations. When the ARS is the sole owner of the cultivar, it has the option of obtaining a plant variety protection certificate or a patent, depending on the species involved. It can grant exclusive rights for distribution of a cultivar to an individual or a company if this is considered necessary to promote use of the cultivar, and can collect a royalty. The revenue would be paid to the U.S. Treasury without any percentage being provided to the ARS or the breeder.

Although the ARS has several options for the distribution of cultivars developed solely by it, the philosophy has been to provide a cultivar to users without remuneration. Plant variety protection has not been obtained for any cultivars, up to the present time.

The most common situation is for the ARS to develop a cultivar in cooperation with scientists from State Agricultural Experiment Stations, including breeders, pathologists, and entomologists. There are no published rules for determining when the ARS is a coowner of a cultivar, but two general principles have been used. ARS is assumed to be a coowner when the ARS scientist is the recognized leader of a cooperative cultivar development program or when the ARS scientist makes decisions that directly influence the development and eventual release of a cultivar.

The ARS and the State Agricultural Experiment Station cooperate as coowners of a cultivar in the decisions concerning its distribution. The ARS will participate in the application for plant variety protection or a patent if initiated by and paid for by the Experiment Station or its designated representative. The details related to seed distribution generally are handled by the Experiment Station.

State Agricultural Experiment Stations. The procedures for distributing cultivars developed by State Agricultural Experiment Stations vary with the type of cultivars involved and the philosophy of the originating institution. In some situations, the distribution is managed by an independent company or corporation that has a contract or mutual understanding with the Experiment Station. The Experiment Station and the breeder may or may not benefit financially from the sale of the genotype. These alternatives will be reviewed by examining procedures used by the Iowa Agriculture and Home Economics Experiment Station at Iowa State University.

Distribution Agencies. The Iowa Agriculture and Home Economics Experiment Station distributes cultivars through two independent corporations closely aligned with the university: the Committee for Agricultural Development and the Iowa State University Research Foundation, Inc. The Committee for Agricultural Development is a nonprofit, nonstock corporation that was formed to assist the Experiment Station in the distribution of new germplasm. As an independent corporation, it can collect, disburse, and invest revenue more readily than the Experiment Station itself. Any revenue received that exceeds the expenses of

the corporation is used to support activities of the Experiment Station. The close relationship between the Committee for Agricultural Development and the Experiment Station is ensured by the makeup of the Board of Directors of the corporation. Four permanent members of the Board are the president of Iowa State University, the dean of the College of Agriculture, the associate director of the Experiment Station, and the head of the Department of Agronomy. Additional members with limited terms on the Board are from outside the University and include farmers and seed producers. The persons employed by the Committee for Agricultural Development to operate the corporation are staff members of Iowa State University.

The Iowa State University Research Foundation is an independent corporation that facilitates the distribution of inventions developed by staff members of Iowa State University. Its Board of Directors include the president and other administrators of the University, and its employees are staff members of the University. Any revenue obtained in excess of expenses is used to support research of the University. It is the policy of the Research Foundation that a percentage of the revenue received from an invention, in excess of the expenses incurred, is given to the inventor. For a patent of a plant cultivar, the inventor would be the breeder who developed it.

Methods of Distribution. *Nonexclusive distribution.* The majority of the cultivars developed by the Experiment Station are distributed by the Committee for Agricultural Development on a nonexclusive basis. A distribution is nonexclusive when seeds or vegetative propagules are sold to a number of individuals or companies that increase and merchandise the cultivars. A royalty could be charged for each unit sold to commercial growers, but that has not been done up to the present time.

Nonexclusive distribution is used for cultivars that can be readily sold by a number of competing individuals or companies. The Committee for Agricultural Development distributes foundation seed of oat and soybean cultivars on a nonexclusive basis. The foundation seed is used to produce certified classes of seed for sale to farmers. Only the Committee for Agricultural Development can distribute foundation seed of oat and soybean cultivars. Therefore, a seed producer who annually produces the registered class of seed must purchase foundation seed from the organization each year.

Inbred lines of maize and sorghum are distributed on a nonexclusive basis by the Committee for Agricultural Development. Individuals and companies can use the inbred lines without restriction for the production of commercial hybrids. They can purify and increase their own seed of the inbred line each year; therefore, they only need to purchase seed of the inbred line from the Committee for Agricultural Development one time.

Committee for Agricultural Development also distributes on a nonexclusive basis germplasm that is intended for use by public and private breeders. This germplasm includes parents for hybridization and populations in which selection could be conducted.

Exclusive distribution. A limited number of cultivars developed by the Experiment Station are distributed exclusively to a single company or corporation by the Iowa State University Research Foundation or the Committee for Agricultural Development. The Foundation manages only the exclusive distribution of cultivars that are patented (App. E). The Foundation obtains a patent for the breeder (inventor) and negotiates a contract with a company for the exclusive distribution of the patented cultivar. Royalties from the company are used by the Foundation to pay for expenses of the patenting process. A percentage of any additional income is shared with the inventor.

Exclusive distribution by the Committee for Agricultural Development has been used for cultivars of forage species that have a limited seed market. A cultivar with a limited market may never be properly distributed on a nonexclusive basis because seed companies could not afford the expense of promoting it in competition with each other. An exclusive distribution was used by the Committee for Agricultural Development for the 'Carroll' cultivar of birdsfoot trefoil. A Memorandum of Understanding was negotiated between the Committee for Agricultural Development (CAD) and a seed company with experience in the distribution of seed of forage species. The Memorandum had the following statement that described the purpose of the exclusive distribution.

> It is the mutual desire of the CAD and the Company to promote the widespread use of Carroll birdsfoot trefoil, a superior variety. Both parties feel that the granting of the exclusive rights for the production, promotion, distribution, and selling of Certified Seed of this variety to the Company is the best means of achieving the goal. It is the belief of the cooperating parties that this promotion will be to their mutual benefit and to the benefit of the people of Iowa.

A royalty was paid to the Committee for Agricultural Development for all certified seed of the cultivar that was sold.

Asexually propagated cultivars that are not patented have been distributed on an exclusive basis by the Committee for Agricultural Development. An agreement was made with the Iowa Nurserymen's Research Corporation for the forsythia cultivar 'Iowa Selection Sunrise.' The Research Corporation is made up of a number of private companies in Iowa that increase and sell plants of asexually propagated cultivars of horticultural species. The Research Corporation agreed to pay a royalty to the Committee for Agricultural Development for each rooted cutting distributed to member nurseries. None of the income from this or any other cultivar distributed by the Committee for Agricultural Development is shared with the breeder who developed it.

LEGAL RIGHT OF OWNERSHIP

In the United States, legal ownership of a new cultivar can be obtained through a plant patent or plant variety protection certificate. The legal owner has the right to control who multiplies and sells the cultivar to commercial growers.

This control provides an opportunity for the originator to profit from its investment in plant breeding.

Plant Patent

A patent can be obtained for asexually propagated plants that are not reproduced by seeds. The cultivar must be distinct from any other cultivar of the species. The commercial value of a cultivar and its performance relative to that of other cultivars are not considerations in granting a patent.

An example of a plant patent is presented in App. E. The person who developed the cultivar is considered the inventor. The assignee is the individual, company, or corporation that has been chosen by the inventor to apply for and administer the patent. The inventor can make the application for a plant patent personally without assigning the responsibility to others.

A plant patent provides the same legal protection as a patent for a machine or design. The inventor or assignee has the legal right to control the multiplication and sale of the cultivar for a specified number of years. Anyone who sells the cultivar without the permission of the patent owner can be prosecuted.

The cost of a plant patent exceeds 1000 dollars. Information about plant patents can be obtained from the Patent and Trademark Office in Washington, D. C.

Plant Variety Protection

Legal ownership of cultivars reproduced sexually by seeds can be obtained by plant variety protection. The inbred lines used to produce a commercial hybrid cultivar can be protected, but not the hybrid per se. The Plant Variety Protection Act was established through Public Law 91-577 approved by the U.S. government on December 24, 1970. Before the law was enacted, it was not possible to obtain legal ownership of seed-propagated cultivars or inbred lines.

The criteria by which a cultivar is evaluated for plant variety protection are novelty, stability, and uniformity. A cultivar must have readily distinguishable characteristics that make it novel from every other cultivar of the species. The novelty statement that describes the distinguishing characteristics is a part of the application form (App. F). After an application is made, the characteristics of the cultivar are compared with those of every other cultivar that has been described for the species, both past and present. If another cultivar is found with the same characteristics, protection will not be granted unless the originator identifies a trait for which they differ. Any qualitative or quantitative character can be used regardless of the commercial value of the trait. A quantitative character, such as yield, can be used only if assurance can be provided that the ranking of a cultivar in comparison with another for that character will not change across environments.

The novelty characteristics of cultivars that have been protected are published quarterly in the *Plant Variety Protection Office Journal* available from the Plant Variety Protection Office; Livestock, Meat, Grain & Seed Division; Agricultural Marketing Service; U.S. Department of Agriculture, National Agricultural Library Building, Beltsville, Maryland 20705. The following examples of novelty were taken from Volume 10, No. 3, July–September 1982.

'Score' cultivar of garden bean developed by Moran Seeds, Inc.

> 'SCORE' is most similar to 'PICKER'; however, the foliage color of 'SCORE' is dark green (Royal Horticultural Society 131B), whereas foliage color of 'PICKER' is yellow-green (RHS 135B). Also, pod color of 'SCORE', is RHS 143C, whereas pod color of 'PICKER' is 143A. Pods of 'SCORE' are borne high on the bush, whereas pods of 'PICKER' are scattered.

'Deltapine 30' cultivar of cotton developed by Delta and Pine Land Co.

> 'DELTAPINE 30' most closely resembles 'DELTAPINE 70'; however, 'DELTAPINE 30' has a higher micronaire reading (5.2 vs 4.8) and weaker fiber (Stl 23.7 vs 24.2 G/Tex) than 'DELTAPINE 70'.

'Southern States 76-30' cultivar of oat developed by Coker's Pedigreed Seed Co.

> 'SOUTHERN STATES 76-30' is most similar to 'COKER 716'; however, 'SOUTHERN STATES 76-30' is 2 or more days earlier and approximately 6 cm taller (100 vs 94 cm) than 'COKER 716'. 'SOUTHERN STATES 76-30' has a hairy lemma dorsal surface, whereas the dorsal surface of lemmas of 'COKER 716' is hairless.

'Orange Cayenne' cultivar of pepper developed by Tommy Bolack.

> 'ORANGE CAYENNE' is most similar to 'CAYENNE LONG RED'; however, 'ORANGE CAYENNE' has bright orange mature fruit, while 'CAYENNE LONG RED' has bright red mature fruits.

'2553' cultivar of common wheat developed by Pioneer Hi-Bred International, Inc.

> '2553' is most similar to 'S76'; however, '2553' is susceptible to Races A, B, C, D, and F of Hessian Fly while 'S76' is resistant. Phenol reaction for '2553' is light brown to brown while the phenol reaction for 'S76' is dark brown to black.

A cultivar is stable that can be reproduced for multiple generations of seed production without changes in its characteristics. It must be uniform to the extent that variants are properly described, predictable, and commercially acceptable.

The originator must specify the role of seed certification in the sale of the cultivar to commercial growers (App. F). One option is to require that all seed be sold by cultivar name only as a class of certified seed. This option prevents anyone from putting a brand name on the seed with the statement "Variety not stated" (App. G). All seed of the cultivar that is sold must have passed the requirements of an official seed certifying agency and the seed container must be labeled accordingly. Another important aspect of the certification option

relates to the prosecution of individuals or companies that merchandise the cultivar without permission of the owner. When the certification option is chosen, the U.S. government has the responsibility to protect the rights of the owner. If an owner believes that someone is selling the cultivar illegally, the U.S. government conducts an investigation and obtains an appropriate settlement, if necessary.

The second option for sale of a cultivar is to permit the sale of either certified or uncertified seed. With this option, the owner can use a brand name and not disclose the cultivar name by use of the statement "Variety not stated." The option to sell certified or uncertified seed makes owners responsible for safeguarding their rights to a protected cultivar. Owners who believe someone is illegally selling seed of their cultivars are responsible for all legal action required to prosecute the offender.

Plant variety protection does not prevent commercial growers from multiplying and planting seed of a cultivar for their own use. Farmers can plant a protected cultivar on their farm and save seed from the harvest to plant the cultivar the next season without paying any money to the owner. A protected cultivar can be used by any individual or company as a parent for hybridization or mutation. It also can be used for research experiments, such as studies on physiology and disease resistance.

Plant variety protection provides an owner with legal rights to a cultivar for 18 years. After that time, the cultivar can be multiplied and sold by anyone as certified or uncertified seed. In emergency situations when the production of food for humans, feed for livestock, or plant material for industry is threatened, the Secretary of Agriculture of the U.S. government can make a protected cultivar available to the public for multiplication and sale.

The application for plant variety protection must be made within a specified time period after the cultivar is commercially available. The fees charged by the Plant Variety Protection Office for protecting a cultivar exceed 1500 dollars. When the initial application is filed, seed of the cultivar may be labeled "Unauthorized propagation prohibited—U.S. Variety Protection applied for." After the application has been examined and a certificate of protection has been issued, seed of the cultivar may be labeled "Unauthorized propagation prohibited—U.S. protected variety."

SEED CERTIFICATION

The seed of cultivars or a planned seed mixture (multiline) of cultivars can be certified for genetic identity and purity by an official seed certifying agency. Each state in the United States independently determines which agency or agencies, if any, will be responsible for seed certification. The agency establishes for the state the requirements that must be met and administers the certification program. Most seed certifying agencies in Canada and the United

States are members of the Association of Official Seed Certifying Agencies (AOSCA).

One of the important activities of AOSCA is to establish minimum standards for genetic purity and identity for the classes of certified seed. No member of the organization may use lower standards for its individual certifying agency, but it may establish higher standards. AOSCA also recommends minimum standards of seed quality, such as germination percentage; however, no member agency is required to include seed quality as a criterion for certification.

Despite the important coordinating role of AOSCA, the voluntary nature of seed certification in the United States makes it difficult to generalize about the system. A company that merchandises seed in several states may encounter different requirements that must be met. All seed certifying agencies must have standards for genetic purity, but the standards for agencies in different states may not be the same. Some agencies require that standards for seed quality must be met for certification. The agencies in some states require that performance data must be obtained by them or their designated representative each year a cultivar or multiline is certified.

To simplify a review of the principles of seed certification, the procedures and standards of AOSCA will be used. Anyone interested in certifying seed in a particular state should become familiar with the procedures and standards used by the appropriate certifying agency.

Cultivars Eligible for Certification

A cultivar or multiline must be approved for certification by at least one certifying agency that is a member of AOSCA or by the National Certified Variety Review Board for the crop. The procedures and requirements of the National Certified Variety Review Boards are representative of those that would be used by individual agencies.

Review boards have been established by AOSCA for alfalfa, grass, small grain, soybean, forage legumes other than alfalfa, and sunflower. Their purpose is to advise member agencies on the eligibility of cultivars for certification. Before the review boards were established, it was necessary for the originator to prepare an application for the agency in each state where the cultivar was to be certified, and each agency had to review the information independently. With the establishment of the review boards, it became possible for an originator to make a single application for eligibility that would apply to more than one state. The member agencies of AOSCA generally accept for certification any cultivar approved by the review board, but are not required to accept the board's decision. If a cultivar is not approved by the review board, the originator may request approval from individual agencies.

The review boards are made up of members from public and private institutions. Organizations represented include AOSCA, American Seed Trade As-

sociation, Crop Science Society of America, National Council of Commercial Plant Breeders, and U.S. Department of Agriculture. The Plant Variety Protection Office is represented by a nonvoting member. The review boards meet at least once annually to review applications.

The application form prepared by the originator is not the same as that used for plant variety protection; however, much of the information requested is similar (App. H). The information includes a statement of the breeding procedures used to develop the cultivar, a description of the cultivar's morphological and physiological characteristics, its reactions to diseases and insects, and procedures for maintenance of seed stocks.

Classes of Certified Seed

The classes of seed in the certification program of the United States are breeder, foundation, registered, and certified (App. I). The originator of the cultivar can specify the number of classes that will be used.

Breeder seed is produced under the direct control of the originator of the cultivar or its designated representative, and is expected to have the highest level of genetic purity of any class of seed. Breeder seed can be used to produce the foundation, registered, or certified classes.

Foundation seed is produced from breeder seed or from foundation seed per se under the control of the originator or its designated representative. Foundation seed can be used to produce the registered or certified classes. In some cases, it is sold to farmers for planting.

Registered seed is produced from breeder or foundation seed. It can be used to produce certified seed, and is commonly used by farmers for commercial planting.

Certified seed is produced from breeder, foundation, or registered seed. It generally is not used to produce additional generations of certified seed, except when foundation seed of an old cultivar is not maintained or in an emergency when supplies of foundation or registered seed are not adequate to plant enough area for certified seed production.

Standards of Genetic Purity

Certification involves inspection of the crop in the field and of samples of the harvested seed. Each field or designated area is considered an independent unit or lot for certification. If seed of a cultivar is produced in 100 separate fields, each field is considered independently for acceptance or rejection. The field and seed inspections are made by employees of the certifying agency.

The requirements that must be met for certification differ for each crop. A field chosen to produce a class of certified seed must meet certain requirements for the types of crops previously grown on it. In general, the land must be free

of volunteer plants of the same species, and no material can be applied to the field before or after planting that may contain seeds or plant parts that would result in the production of off-type plants. The field must be properly isolated from sources of pollen or seed that would cause a reduction in genetic purity.

The percentage of off-type plants that can be present varies with the crop species and the class of seed being produced. The maximum percentages of off-type plants that can be present in rice are 0.01 for foundation, 0.02 for registered, and 0.1 for certified. The maximum percentages for peanuts are 0.1 for foundation, 0.2 for registered, and 0.5 for certified.

Samples of seed are inspected from each field. Standards of genetic purity are established for each crop species and class of seed. The maximum percentages of off-type seeds for cotton are 0.03 for foundation, 0.05 for registered, and 0.1 for certified. The maximum percentages for barley are 0.05 for foundation, 0.1 for registered, and 0.2 for certified.

Seed Conditioning

The cleaning and preparation of seed for distribution, referred to as conditioning, only can be done by individuals or companies that are approved by a certifying agency on an annual basis. Approved conditioners must have facilities that can condition seed without introducing mixtures. They must be able to maintain the identity of seed and keep records on its disposition.

Seed Labeling

The bag or container in which a class of certified seed is sold must have an official certification label attached to it. The labels must meet the specifications of the certifying agency. The colors commonly specified for the classes of seed are white for foundation, light purple for registered, and light blue for certified. The label contains the name of the certifying agency, an identification number for the lot of seed, the cultivar name, the crop, and the class of seed (App. I).

Certification of Cultivar Mixtures or Blends.

Planned seed mixtures (multilines or blends) of cultivars are certified by some agencies. The following are the 1985 requirements of the Iowa Crop Improvement Association.

Certification of Varietal Blends

a. A blend to be certified shall be approved by the Board of Directors.
b. Permission to use a protected or private variety in a blend must be obtained from the breeder or owner of the variety. This evidence must be submitted by the blender to the certifying agency.

c. Each year that a blend is offered for sale, current performance data from tests conducted by or for the Association the previous year shall be available to the purchasers of seed. A blend shall be tested at two or more locations in its area of adaptation in comparison with appropriate check varieties. The fee for testing shall be paid by the breeder, sponsoring organization or institution.

d. A given component of a blend shall be not less than 15 percent of the whole on a weight basis. The sampling tolerance limits of each component shall not exceed plus or minus 10 percent of the whole also on a weight basis. No more than four components (varieties) may be used in a given blend.

e. The components of a blend must have met all field and seed requirements for certification previous to blending.

f. Only Approved Seed Conditioners may blend varieties to be sold as certified seed.

g. The Approved Seed Conditioner shall demonstrate the ability to blend varieties within specified tolerances.

h. The size of a given lot of a given blend shall not exceed 3000 bushels.

i. The components and their proportions within a blend shall be recorded in the files of the Iowa Corp Improvement Association, and this formula shall not vary between lots and between years. Closed formulas will be retained in confidence.

The blendor has the option of stating or not stating the name of the varietal components and proportions on the label provided there is compliance with state and federal laws.

j. Any additional expenses incurred for sampling, testing, etc. in the certification of blends shall be paid for by the Approved Conditioner making the blend.

k. The certification tag shall carry the following information:

(1) *Protected varieties* (as required by owner)

Iowa Certified Seed

Blend and Kind _____

Component A % by wt. _____ % germ. _____

Component B % by wt. _____ % germ. _____

Component C % by wt. _____ % germ. _____

Lot No. _____

Approved Seed Conditioner No. _____ affirms that all the varieties in the blend of seed to which this tag is attached have passed field and seed certification requirements of an official seed certifying agency. Tag Serial No. _____

(2) *Non-protected varieties* or protected varieties where owner does not require listing of components on tag.

Iowa Certified Seed

The Approved Seed Conditioner listed below affirms that the seed to which this tag is attached is a blend of varieties that have passed field and seed certification requirements of an official seed certifying agency. VARIETIES NOT STATED

Blend and Kind _____

Lot Number _____

Approved Seed Conditioner Number _____

Tag Serial No. _____

REGULATORY AGENCIES

The distribution of seed in the United States is controlled by federal and state laws. The general nature of the regulations will be examined by considering those established by the federal government. The information is taken from five pamphlets entitled *Federal Seed Act: Interstate Commerce, Foreign Commerce, and General Regulations; Seed Testing Regulations; Certified Seed Regulations;* and *Rules of Practice*. Copies are available from the Seed Branch, Grain Division, Agricultural Marketing Service, U.S. Department of Agriculture, Washington, D. C. 20250.

The Federal Seed Act was approved on August 9, 1939, and has been amended when necessary by the U.S. Government. The purpose of the Act is to "to regulate interstate and foreign commerce in seeds; to require labeling and to prevent misrepresentation of seeds in interstate commerce; to require certain standards with respect to certain imported seed; and for other purposes."

One important responsibility of the Act is to regulate the information that must be provided about the seed that is being distributed and merchandised in interstate commerce. Each container must have a label with appropriate information, some of which is the following:

1. The name of the crop species and the name of the cultivar for each seed component present in excess of 5 percent of the whole and the percentage by weight of each component. The name of the cultivar can be replaced by the statement "Variety not stated," except when a certificate of plant variety protection specifies that it only can be sold by variety name as a class of certified seed.
2. Location where the seed was produced.
3. Percentage by weight of weed seeds.
4. Percentage by weight of seeds of other crops.
5. Percentage by weight of inert matter.
6. Germination percentages of appropriate components of the seed, and the calendar month and year the test for germination was completed.

State seed laws may vary from that of the Federal Seed Act. For example, the Federal Seed Act permits use of the statement "Variety not stated" as a substitute for the cultivar name, unless prohibited by a certificate of plant variety protection. The seed laws of some states require that the variety name always be stated, and prohibit use of the statement "Variety not stated."

Personnel from an appropriate federal or state agency are responsible for enforcing the seed laws. Periodic checks of seed are made to ensure that the laws are being properly followed. Violation of the laws can prevent the sale of seed and may result in financial penalties.

Appendixes

APPENDIX A

The National Plant Germplasm System

Plant germplasm is the base for productive agriculture. It is the genetic raw material required by breeders for the development of new, superior crop varieties that can ensure a stable, plentiful supply of food, feed and fiber having desirable qualities. Acquisition, preservation, evaluation, and distribution activities of U.S. germplasm resources are coordinated by the National Plant Germplasm System (NPGS).

The NPGS is designed to provide, on a continuing, long-term basis, the plant genetic diversity needed by farmers and public and private plant scientists to improve productivity of crops and minimize the vulnerability of those crops to biological and environmental stresses. Genetic vulnerability of crops comes into play when an out-of-the-ordinary range of stresses from diseases, insects, drought, or temperature extremes exceeds the crop's range of tolerance or resistance to such factors. The results can vary from noticeable yield reduction in localized areas to disastrous crop failures over very large areas.

Protection from crop losses through control of biological and environmental stresses is far more difficult and costly than through increased genetic diversity among varieties of a given crop. Therefore, a NPGS objective is to broaden the genetic diversity of a crop throughout its production area by having that production come from an array of varieties, all productive but each different from the others in its range of tolerance to one or more potential stresses. This variety and range can reduce the likelihood of epidemic losses.

The NPGS now maintains over 400,000 accessions of germplasm in the form of seed and vegetatively propagated stocks. These accessions are primarily landraces and unimproved germplasm from foreign sources. A few working collections and the National Seed Storage Laboratory (NSSL) also maintain some domestic breeding lines and cultivars. Any of this wide array of genetic diversity is available without charge to any *bona fide* plant scientist in the United States. In addition, material in the NPGS is exchanged

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with countries around the world for germplasm needed by U.S. scientists. In providing germplasm to users, domestic or foreign, only a portion of a given accession leaves the system. A given accession is never exhausted—it is maintained and increased as necessary.

New accessions of germplasm are added to the system at the rate of 7000 to 15,000 per year. Approximately 70 to 80 percent of these come through exchange with other countries; the rest are acquired directly through foreign and domestic collecting expeditions, and from the user community.

Management of the diffuse system is largely delegated through USDA with the primary coordinating function residing with the Assistant to the Deputy Administrator for Germplasm within the Agricultural Research Service (ARS). The NPGS is also a major component of an international plant germplasm network and, as such, coordinates its efforts with the International Board for Plant Genetic Resources (IBPGR).

The major activities of the NPGS are acquisition, maintenance, evaluation, and enhancement of plant germplasm; research on conservation of genetic diversity; monitoring genetic vulnerability; and information management. These activities are carried out at various locations in the U.S. and its territories. In addition to the thousands of individual federal, state, and private scientists who do research involving breeding, evaluation, and improvement of germplasm, there are four major structural components that make up the NPGS. These are (1) plant introduction facilities and activities; (2) collections—maintenance and evaluation facilities and activities for the long-term base collection at the National Seed Storage Laboratory and the various working collections that exist in federal, state, and private organizations; (3) an information system used for management and operation as well as enhanced communication to scientists regarding the location and characteristics of germplasm they may wish to obtain for research purposes; and (4) various advisory groups which represent the federal, state, and private organizations in the NPGS and advise on germplasm issues as well as specific crop issues and technology.

As an introduction to the National Plant Germplasm System, these structural components and several of their individual units or organizations are described in this overview.

Plant Introduction

The Plant Introduction Office (PIO) is part of the *Plant Genetics and Germplasm Institute* (PGGI) of USDA/ARS at Beltsville, Maryland. This office is the focal point for the acquisition and exchange of plant germplasm. It catalogs all incoming accessions, assigns plant inventory (P.I.) numbers, and distributes P.I. material to maintenance centers or curators according to established protocols and priorities. No collections are maintained by this office.

The Plant Taxonomy Laboratory (PTL), PGGI, identifies material entering the NPGS and provides correct scientific nomenclature. It also plans, expedites, and participates in plant exploration.

The Economic Botany Laboratory (EBL), PGGI, undertakes studies to determine the geographical and ecological distribution of significant diversity in crop species.

The Plant Introduction Station at Glenn Dale, Maryland, is also part of the PGGI. This station distributes pest-free material of prohibited and post entry quarantine categories

of fruits, woody ornamentals, and certain vegetables. It also serves as the Plant Quarantine Facility of the USDA Animal and Plant Health Inspection Service (APHIS).

The Plant Introduction Station at Miami, Florida, is an integral part of the Subtropical Horticultural Research Station of USDA/ARS. This station maintains, evaluates, and releases new varieties of mango, jujube, avocado, and other tropical and subtropical fruits and provides disease-free maintenance of coffee and cacao.

The four state/federal *Regional Plant Introduction Stations* (RPISs) at *Geneva, New York* (NE-9), *Experiment, Georgia* (S-9), *Ames, Iowa* (NC-7), and *Pullman, Washington* (W-6), each have priority responsibility for maintaining primarily "wild type" and introduced germplasm of many selected crops. The crop responsibility lists may include not only crops maintained at the RPIS but also those under other curators at outlying locations in the region. Should any of the outlying collections come under any jeopardy, it is the responsibility of the Regional Coordinator at the RPIS to take steps that will assure their continued safe maintenance. The Coordinators (all are federal) have a national responsibility for each species assigned to them. Some of the major crop responsibilities of each station are as follows:

- *Northeastern Regional Plant Introduction Station, Geneva, New York:* Perennial clover, onion, pea, broccoli, and timothy.
- *Southern Regional Plant Introduction Station, Experiment, Georgia:* Cantaloupe, cowpea, millet, peanut, sorghum, and pepper.
- *North Central Regional Plant Introduction Station, Ames, Iowa:* Alfalfa, corn, sweet clover, beets, tomato, and cucumber.
- *Western Regional Plant Introduction Station, Pullman, Washington:* Bean, cabbage, fescue, wheat, grasses, lentils, lettuce, safflower, and chickpeas.
- *State/Federal Interregional Potato Introduction Station (IR-1), Sturgeon Bay, Wisconsin:* Focuses on potato variety development with strong emphasis on germplasm maintenance and upgrading to meet breeders' needs. It also supports research on methods for effective maintenance of potato germplasm in the form of clonal material, either through tuber regeneration or meristem preservation.

Collections

The National Seed Storage Laboratory (NSSL) at Fort Collins, Colorado, is a USDA/ARS facility and the nation's only long-term seed storage facility. It has been in operation since 1958. The Laboratory maintains plant germplasm as a *base collection* for the United States and is a backup base collection for many crops in support of the global network of genetic resources centers. Present categories of stocks in storage include basic plant introductions, recently released and obsolete varieties, open-pollinated parental lines, genetic stocks, differential host and virus indicator stocks, and type specimens of varieties registered under the Plant Variety Protection Act for future reference.

The NSSL base collection is not intended to meet the day-to-day needs of plant breeders and other plant scientists, but rather serves as a reserve stock to prevent loss of germplasm and erosion of genetic diversity. Generally, seed samples in the base collection are also held in a working collection outside the NSSL and therefore are distributed from the NSSL only when unavailable from another source.

Base collection samples are for indefinite storage with regrowing as infrequently as

possible so that genetic changes through repeated seed increases do not occur. However, the seed is regrown often enough to prevent loss of viability. The working collections are analogous to checking accounts—there to be drawn upon as needed, but not overdrawn. The base collection is analogous to a savings account—to be used only when the checking account runs out.

The NSSL's research is concentrated on determining optimum storage conditions for each group of species with similar storage requirements. Seed viability is monitored on a regular schedule. The Laboratory has a current inventory of over 200,000 accessions.

The primary objective of the *National Clonal Repositories* is to maintain and preserve valuable fruit, nut, and other selected crops which are normally propagated by vegetative means and to make such germplasm readily available to plant breeders and other plantsmen. Secondary objectives of the repositories are to collect, worldwide, accessions of valuable germplasm; to evaluate such accessions; and to conduct and encourage appropriate research related to improved methods of evaluation, propagation, preservation, storage, and distribution of clonal germplasm. Twelve separate clonal repositories are planned. Five are now in operation:

- *Corvallis, Oregon:* Pears, filberts, hazelnuts, small fruits, hops, and mint.
- *Davis, California:* Grapes, stone fruits, and nuts.
- *Miami, Florida:* Some subtropical and tropical fruits and sugarcane.
- *Indio, California:* Date palm.
- *Mayaguez Institute of Tropical Agriculture (MITA), Mayaguez, Puerto Rico:* Tropical fruits and industrial crops.

The USDA Small Grains Collection is located in the *Plant Genetics and Germplasm Institute* (PGGI) at Beltsville, Maryland. This working collection contains some 90,000 wheat, barley, oats, rice, rye and *Aegilops* accessions. Annually, over 100,000 samples of these accessions are distributed in response to requests from all parts of the world.

Working collections are an assemblage of germplasm (genetic resources) maintained to meet the day-to-day research needs of breeders, geneticists, pathologists, entomologists, cytologists, agronomists, horticulturists, and other users who wish to utilize it for research purposes. The curators of working collections provide the primary interface with the user community. Requests for seed are channeled through the curators and seed from domestic sources enters the system through the working collections. Working collections include foreign acquisition, wild relatives of crop species, acquisitions from the domestic flora and domestic cultivars, plus some advanced lines recommended by the NPGS Crop Advisory Committees (see p. 475). It is a goal of NPGS that all accessions in working collections also be catalogued and maintained in the National Seed Storage Laboratory.

A *curator* is an individual who has accepted specific responsibility to physically maintain, protect, control access to, and distribute specific plant germplasm. An individual curator may be the coordinator of a RPIS or someone independent of the RPISs but in an identifiable curatorial position.

The curators of these working and base collections agree to maintain the collections under good storage conditions and by seed rejuvenation as required, or by protected, well-managed plant repositories in the case of clonally propagated species. Curators maintain a current inventory of accessions in the collection and agree to make reasonable amounts of the germplasm under their care available at no charge to *bona fide* research scientists and institutions. The curator does not have the option of discarding elements

of the collection on his own volition. When changes in program, personnel, physical facilities, or administrative policy place a collection in jeopardy, it is the curator's responsibility to notify appropriate officials within the NPGS.

Another important portion of the NPGS, which falls in the germplasm collection category, pertains to the *genetic and mutant stock centers*. These are working collections of individual accessions genetically defined by a specific genetic or chromosomal trait controlled by a gene at an allele, locus, chromosome, translocation, inversion, and so on. The genetic stock centers are an essential underpinning of the research effort, both basic and applied, on plants in the United States and throughout the world. These stocks have been utilized for research and education in plant breeding, genetics, physiology, biochemistry, and molecular genetics. More specifically, advances in knowledge made possible by, or at least facilitated by, the existence of these stock centers, have been in the areas of: gene function and process of mutation; fine structure of genetic material; behavior and mechanisms of chromosomes; processes of starch biosynthesis; biosynthesis of storage proteins and carotenoids; and existence and properties of migrating genetic materials and mutable loci. These genetic and mutant stocks often require specialized maintenance procedures.

Examples of notable genetic stock collections are

Barley: Over 3000 genetic stocks, maintained by and distributed from the Department of Agronomy, Colorado State University, Fort Collins.

Cotton: About 300 genetic stocks, maintained by and distributed from the Agronomy Field Laboratory, Texas A&M University, College Station.

Oats: Over 200 genetic stocks, maintained by and distributed from the Small Grains Collection, ARS, Beltsville, Maryland.

Peas: 5000 single mutants, genetic stocks, multimarker lines, linked genes, maintained by and distributed from the Department of Seed and Vegetable Sciences, New York State Agricultural Experiment Station, Geneva.

Corn: About 51,000 different genotypes, in addition to translocations and other chromosome or cytological stocks, are maintained and distributed from the Maize Genetics Cooperation Stock Center, Department of Agronomy, University of Illinois, Urbana.

Tomatoes: 1700 genetic and chromosomal stocks of *Lycopersicon esculentum* and related species, maintained by and distributed from the Department of Vegetable Crops, University of California.

Wheat: 600 genetic stocks, maintained by and distributed from ARS scientists at the University of Missouri, Columbia.

Information System

A feasibility study was conducted during 1976–77 which investigated and identified the need for information management systems in the efficient collection, conservation, distribution, and utilization of plant germplasm in the National Plant Germplasm System.

The USDA Science and Education/Agricultural Research Service recognized the critical need for a nationally unified information system to serve the diverse needs of the NPGS. A cooperative agreement with the Laboratory for Information Science in Agri-

culture (LISA) to develop a computer-based information system led to formation of the Germplasm Resources Information Project (GRIP).

Analysis of the diverse needs of the NPGS community, its abundant information resources, and the necessary management and use of those resources led to identification of two basic groups of information users within the NPGS—those who supply and those who demand information. "Suppliers" are those who *acquire*, *maintain*, and *distribute* germplasm and data such as curators, and staff of the NSSL and various plant introduction stations. The "demand" group is composed of those who *use* germplasm and data such as plant breeders, scientists, and researchers. The needs of both groups were identified and small-scale operational prototypes of the system were developed and installed at such NPGS sites as the Regional Plant Introduction Stations. Testing and evaluation of these prototypes (including consideration of user responses and suggestions) then led to the information system's user-oriented design. Among the ways the information system will serve the supply side will be by providing mechanisms or tools to register accessions as they enter the NPGS, maintain seed inventories, monitor viability of collections, process seed orders, exchange information with other "suppliers," and generate summary reports. The system will allow the demand side to receive information on accessions (including characteristic data and use, and location in the system) as well as requested samples on a timely basis.

The now completed design phase and start of a four-phased implementation will bring the transformation of GRIP to GRIN—the Germplasm Resources Information Network—and continued growth of information management and use in the NPGS.

The Information Network will include not only computer hardware and software, but also people performing specialized tasks, work procedures, and administrative and policy functions. GRIN has been designed to accommodate growth of the NPGS and changing needs brought about by that growth—including additional information system features and more NPGS facilities and users. This flexibility in fulfilling many critical needs is the key to GRIN's anticipated success and continued evolution.

Advisory Groups

The National Plant Genetic Resources Board (NPGRB) provides policy advice directly to the Secretary of Agriculture. The task of the Board is to advise the Secretary on problems, needs, and welfare of the nation's plant genetic resources activities as these impact the food production system.

The duties of the National Plant Genetic Resources Board are: (1) to inform themselves of domestic and international activities to minimize genetic vulnerability of crops; (2) to formulate recommended actions and policies on collection, maintenance, and utilization of plant genetic resources; (3) to recommend actions to coordinate the plant genetic resources plans of several domestic and international organizations; (4) to recommend policies to strengthen plant quarantine and pest monitoring activities; and (5) to advise on new and innovative approaches to plant improvement.

The Board meets at least twice each year. Members of the Board are appointed by the Secretary.

The National Plant Germplasm Committee (NPGC). This Committee was established on May 20, 1974, when the Agricultural Research Service (ARS) agreed to a restructuring of the National Coordinating Committee for New Crops, which had been created in 1949

by State Agricultural Experiment Station (SAES) directors. The functions of the NPGC are

- Provide coordination for the research and service efforts of federal, state, and industry units engaged in the introduction, preservation, evaluation, and distribution of plant germplasm, through representation of all units' views by Committee members.
- Develop policies for the conduct of the national plant germplasm program and for its relationships to international plant germplasm programs.
- Develop research and service proposals and justification for adequate funding of regional and national plant germplasm activities.
- Actively advocate mutually agreed upon proposals with SAES associations and USDA agencies.
- The NPGC forum will also be the principal way in which SAES interests can be presented and harmonized with federal interests at a technically informed level.

The NPGC is chartered to meet at least once each year.

Each of the four regions (NE-9, NC-7, S-9, W-6) has a *Regional Technical Committee* composed of a representative from each State Agricultural Experiment Station in the region as well as representatives from Agricultural Research Service and Soil Conservation Service and, in some cases, Forest Service and Bureau of Land Management (Department of Interior). Each regional technical committee has an Administrative Advisor who is a State Agricultural Experiment Station director. The committees provide technical advice to the Regional Plant Introduction Stations and make policy recommendations to the National Plant Germplasm Committee. Each committee is represented on the NPGC by its respective Administrative Advisor.

The *Crop Advisory Committees* represent the germplasm user community and provide guidance and coordination to the NPGS. There are currently 13 committees—one each for alfalfa, barley, beans, corn, cotton, oats, peanuts, peas, potatoes, sorghum, soybeans, tomatoes, and wheat. The crop advisory committees are composed of plant scientists drawn from the public sector, both the federal and state, as well as from the private sector. The curator of each crop serves as a member on his specific crop's committee. The crop advisory committee provides both general and specific guidelines, policy and work programs, for work and activities in the germplasm management of a specific crop.

The crop advisory committees have worked on problems regarding exchange of information and have developed minimum lists of descriptors to characterize each crop. They have also developed germplasm evaluation plans. Other pertinent issues addressed by the committees are

- Germplasm acquisition strategies.
- Working collection storage conditions.
- Long-term storage conditions.
- Regeneration.
- Seed distribution guidelines.
- Standards for germplasm evaluation.

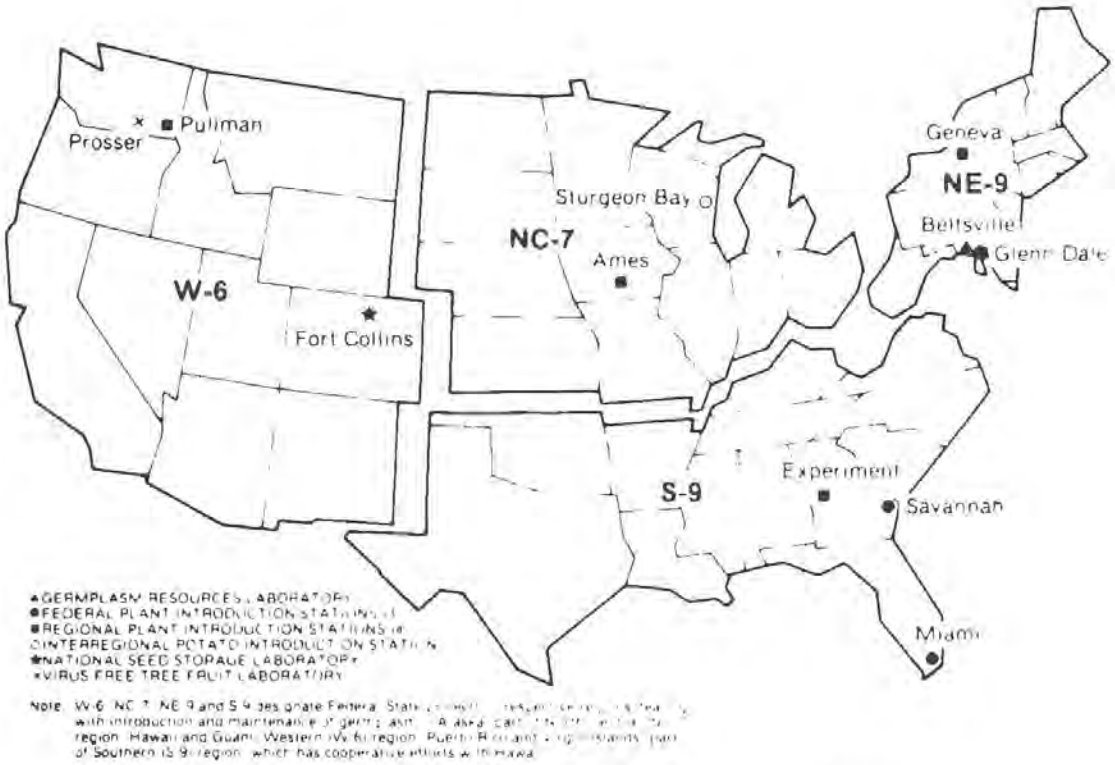
The ARS Plant Germplasm Coordinating Committee coordinates day-to-day operational matters; advises the Administrator of ARS of problems, needs, and opportunities; and recommends priorities for ARS-funded explorations.

The International Board for Plant Genetic Resources (IBPGR) is an autonomous,

international, scientific organization under the aegis of the *Consultative Group on International Agricultural Research* (CGIAR). The IBPGR, which was established by the CGIAR in 1974, is composed of 15 members from 13 countries; its Executive Secretariat is provided by the Food and Agriculture Organization (FAO) of the United Nations, Rome.

The basic function of the IBPGR, as defined by the CGIAR Group, is to promote an international network of genetic resources centers to further the collection, conservation, documentation, evaluation, and use of germplasm and thereby contribute to raising the standard of living and welfare of people throughout the world. The CGIAR Group mobilizes financial support from its members to meet the budgetary requirements of the International Board.

The International Board's network includes regional, national, and international institutions working to preserve the world's dwindling genetic resources. Among these institutions are International Agricultural Research Centers of the CGIAR and other institutions which have agreed to IBPGR designation as those responsible for maintaining major base (i.e., long-term storage) seed collections of the world-wide principal food crops. There are a limited number of designated base collections, because their operation requires extensive facilities and labor. For this reason several base collections are in developed countries. However, material in these collections is freely available and it is the International Board's policy to encourage and assist the establishment of genebanks in centers of genetic diversity (which are almost exclusively developing countries). These locations provide advantages to genebanks in terms of multiplication, evaluation, regeneration, and regional quarantine restrictions.



Major stations of the National Plant Germplasm System.

APPENDIX B

A Statement of Responsibilities and Policies Relating to Development, Release, and Multiplication of Publicly Developed Varieties of Seed-Propagated Crops

A policy statement of the Experiment Station Committee on Organization and Policy of the Experiment Station Section of the Association of State Universities and Land Grant Colleges, and the Agricultural Research Service and the Soil Conservation Service of the United States Department of Agriculture.

June 26, 1972

Foreword

This policy statement pertaining to development, release, and multiplication of varieties is intended for guidance of the State Agricultural Experiment Stations and the United States Department of Agriculture. In this policy statement the term *variety* (synonymous with the term *cultivar*) is used in accordance with the International Code of Nomenclature of Cultivated Plants, 1969.

The correctness of use of the terms "cultivar" and "variety" in the English language is frequently not clearly understood. The International Code of Nomenclature of Cultivated

Plants has adopted the term "cultivar" as an international term which is proper for use in any language. In the English language, the term "variety" may be used as an exact equivalent or as a synonym of "cultivar." Care should be taken not to confuse the term with the English translation of *varietas*, also *variety*, which is a botanical classification. To insure differentiation between "variety" when used for a cultivated variety and "variety" when used as a botanical classification, the abbreviation of the former is cv., whereas the abbreviation for the latter is var.

In the English language version of editions of the Code prior to 1969, the term "variety" was included in parentheses throughout the Code following each use of the term "cultivar." This medium was decided upon so that no one could possibly question the complete equivalence of the terms "cultivar" and "variety" when referring to cultivated varieties. The redundancy of repeating both terms was eliminated in the 1969 edition by the International Commission for the Nomenclature of Cultivated Plants. Instead, the following explanations were included:

> The term cultivar is equivalent to variety in English, *variete* in French, *variedad* in Spanish, . . . whenever these words are used to denote a cultivated variety.

Article 10, Note 4:

> The terms cultivar and variety (in the sense of cultivated variety) are exact equivalents. In translations or adaptations of the Code for special purposes, either *cultivar* or *variety* (or its equivalent in other languages) may be used in the text.

Clearly, the 1969 edition in no way represents a change in policy relative to use of the English term "variety." In fact, if the Code were to be reproduced for popular use in the English language, the International Commission would sanction use of only the term "variety" throughout the entire Code. There certainly is no regimentation in the Code for universal use of the term "cultivar" when referring to cultivated varieties.

It would seem that good judgment should prevail in the use of the equivalent terms. In scientific papers which have international consumption, the international term "cultivar" may be most clearly understood. In papers or documents intended for use by the English-speaking lay public or nonscientific community, the term "variety" may often be considered the more desirable synonym.

The term "variety" means a subdivision of a kind which is distinct, uniform, and stable: "distinct" in the sense that the variety can be differentiated by one or more identifiable morphological, physiological, or other characteristics from all other varieties of public knowledge; "uniform" in the sense that variations in essential and distinctive characteristics are describable; and "stable" in the sense that the variety will remain unchanged to a reasonable degree of reliability in its essential and distinctive characteristics and its uniformity when reproduced or reconstituted as required by the different categories of varieties. The definition of a variety is understood to include the following categories: clonal varieties, line varieties (inbreds), open-pollinated varieties of cross-fertilizing crops, synthetic varieties, hybrid varieties (F_1), and F_2 varieties.

This policy statement has been developed with full cognizance of the contents and implications of the Variety Protection Act, Public Law 91-577. Mutually helpful working relationships among the State Agricultural Experiment Stations, the United States Department of Agriculture, and private plant breeders and seed companies should be encouraged to enhance the effectiveness of both public and private plant breeding efforts.

This revision of the policy statement (dated June 26, 1972) supersedes all previous

documents. It has been approved in the four State Agricultural Experiment Station Director's Associations, The Experiment Station Committee on Organization and Policy (ESCOP), and the Agricultural Research Service and the Soil Conservation Service of the USDA.

This statement outlines general policies and procedures and points up general functions and opportunities for improving both public and private activities and services in the development and use of improved seeds and other propagation materials of publicly developed varieties. It covers seed-propagated varieties of both field and horticultural crops. Adaptations to specific crops will be required.

State Agricultural Experiment Stations (SAES) and the U.S. Department of Agriculture (USDA) were established to serve farmers, industries related to agriculture, and through these, all the people. SAES and USDA have functions and responsibilities at local, state, regional, and national levels. Both are supported largely by public funds. The public interest and good judgment require that they work together and reduce duplication to the desired minimum. Close cooperation in developing policies for making results of individual and joint effort available to the public is an obligation. This includes policies concerned with developing and distributing improved crop varieties from state and federal plant breeding operations, and also working with and assisting private enterprise to serve the public effectively.

A statement of important points of policy in developing improved varieties and releasing these to seed producers and seed users follows:

1. Sources for New Germplasm[1] Improvement

(a) *Collection, introduction, and preliminary evaluations of new plant germplasm*

The USDA, through its Agricultural Research Service, in cooperation with the State Stations and the SCS National Plant Materials Center, collects, distributes, and preserves plant germplasm from foreign and domestic sources. Through various cooperative arrangements, plant characteristics are determined and catalogued. These include reactions to insects, diseases, and climatic variations and determination of quality, potential promising end-products, and other desirable traits. This information is made available to public and private agencies.

State and other federal agencies also conduct domestic and foreign plant explorations. Such activities should be coordinated with those of the Agricultural Research Service in order to eliminate possible duplication in germplasm originally introduced and its subsequent evaluation and distribution. Provision to make resulting plant collections available to public and private plant breeders is encouraged.

Breeding lines and nonreleased varieties received from cooperating scientists, domestic and foreign, should be handled in a manner that will not violate the terms or conditions under which they are obtained.

(b) *Use of introductions*

As a further source of information on the characteristics of introductions, reports on observation and performance tests are requested from those receiving the materials. These reports are compiled, annotated, and disseminated through the four regional research (RRF) projects on new crops.[2] Lists of stocks preserved in the National Seed Storage Laboratory, Fort Collins, Colorado, are prepared and distributed. Individuals or organizations proposing

[1]Germplasm is defined as the material basis of heredity. The one-word format has been adopted. (*Dictionary of Genetics*, R. L. Knight, Chronica Botanica Company, Waltham, Massachusetts, 1948.)

[2]Reference: The National Program for Conservation of Crop Germplasm. *A Progress Report on the Introduction, Screening and Preservation of Plant Material, June 1971*. University of Georgia, Athens, Georgia.

to increase and distribute seed or plant materials of such introductions in their original genetic form are asked to make this intention known to the agency from which the material came. Plans for joint release, thereby, can be considered. Confusion that might arise from duplication of identifying names or numbers given to the same introduction by public or private interests can thus be avoided (see section 5).

(c) *Recognition of originating source of introduced materials*

The source of introduced plant materials should be publicly acknowledged. Original Plant Introduction (PI) number or other identifications should be cited.

When the genetic make-up of the introduced material is modified by selection, inbreeding, or hybridization, and the value of the line has been demonstrated as a new variety, as a breeding line, or as the source of a specific genetic character, the agency providing the original material should be informed of the specific characters in the new variety or line derived from the original introduction. The original source of these breeding materials should be acknowledged publicly, again referring to the PI number, or to an identifying accession number when no PI number has been assigned.

2. Studies of Heredity and Methods of Improvement

(a) *Obligation of State Agricultural Experiment Stations and U.S. Department of Agriculture*

The SAES and USDA are obligated to conduct studies of the characters and properties of plant materials, modes of reproduction, the inheritance of characters, and the possibilities of modification and control of heredity.

(b) *Prompt availability of results*

These agencies and their workers are further obligated to make the results of these studies available to all plant breeders, public or private, through prompt publication of research findings.

(c) *Availability and use of basic genetic materials*

Basic genetic materials should generally be released to all plant breeders who request them. The term *basic genetic material* refers to plant material possessing one or more potentially desirable characters which, in the opinion of the Experiment Station Directors and or agency Administrators, may be of value in plant breeding and when, in their opinion, such general release is in the best interests of United States agriculture and the state or agency research program.

Periodically, the originating station and/or agency should notify the public of germplasm releases, specifying limitations on use and on the amount of material available for distribution.

Every effort should be made to insure that basic genetic materials are not monopolized by any interests. Furthermore, inbreds, experimental lines, and basic genetic materials should not be released in foreign countries prior to their release in the U.S., unless it is agreed that there is little prospect of the material being of value in this country.

(d) *Acknowledgment of use of publicly or privately developed basic genetic materials*

Public acknowledgment of the use of publicly or privately developed basic genetic materials in the development of a new variety is an obligation of the recipient agency, industry group, or individual, as it gives due recognition to the contribution by public or private programs.

3. Breeding to Develop Superior Varieties

(a) *A function of the Stations and the U.S. Department of Agriculture*

The breeding of better varieties to reduce production hazards, to improve quality, and to increase biological efficiency is one of the important functions of the State Stations and the Department. As problems arise which can be solved by plant breeding, it is obvious that these governmental agencies have an obligation to investigate them.

(b) *Interrelations with private plant breeding programs*

Free interchange of a wide range of materials, specialized facilities, scientific competence in many disciplines, and the opportunity to test, observe, and study reactions under a wide range of environmental conditions enhance the probability of success.

(c) *Acknowledgment of use of publicly and privately released germplasm*

Public acknowledgment of the use of publicly and privately released germplasm in a closed-pedigree variety is an obligation of the recipient agency, industry group, or individual, as it gives due recognition to the contribution by public or private programs.

4. Testing and Evaluating Experimental Varieties

(a) *Adequate comparisons with standard varieties*

Experimental varieties and lines should be tested for yield, quality, survival, disease and insect reaction, and other important characteristics in comparison with standard varieties, using techniques that assure valid measures of performance.

(b) *Interstate and regional tests*

Some varieties are not limited in adaptation by local, state, regional, or national boundaries. Interstate testing and interchange of materials should be encouraged. When appropriate, international testing should also be encouraged. Regional testing facilitates more general use of widely adapted varieties. It also reduces time needed to provide reliable information on varietal adaptations.

(c) *Testing for special requirements*

New varieties of crops to be used for food should be tested for those components of nutritive composition or concentration of toxic constituents in which they reasonably might be expected to vary significantly from varieties in commercial production. The term "vary significantly" has been tentatively defined as varying 10 percent in toxicological content and 20 percent in nutritive content. The Food and Drug Administration, HEW, requires submission of data for proposed new food varieties that have had significant alteration of such composition. Submitted data will permit determination as to whether the variety merits listing as "Generally Regarded as Safe" (GRAS). (Federal Register, Document 71-8976, page 12094, June 18, 1971.)

New varieties of crops to be used for specialized industrial or other purposes should be tested for these uses to insure that they are satisfactory. The trade, industry, and specialists using the crop should have opportunity to evaluate a variety before it is released.

(d) *Protecting lines and varieties against premature or unauthorized distribution*

All reasonable precautions should be taken to protect the privileged or restricted status of propagating materials, experimental lines, or experimental varieties during testing and seed increase to prevent pirating and premature or unauthorized distribution prior to release. The possibility that an application for variety protection may be filed intensifies the need for such precaution.

5. Decisions on Release of Varieties

(a) *Policy committee or board of review for variety release*

Decisions on the release of new varieties should be made for each state by the appropriate agricultural agency of that state. It is recommended that in each state there be a policy committee or board of review charged with the responsibility of reviewing the proposal for the release of a new variety. Appropriate information concerning characteristics, performance, area of adaptation, specific use values, seed stocks, and proposed methods of varietal maintenance and increase and distribution should be presented to this committee as a basis for its decision.

(b) *Interstate release procedures*

When a variety has been tested on an interstate basis, opportunity to consider simultaneous release should be given each state in the interstate program.

If, for some reason, prior interstate testing was neglected or impossible, the state which may shortly release a new variety should offer to all interested states seed of the new variety for testing and increase. Nearby states may thus obtain information to answer questions from potential users about the new variety. Regional advisory committees may set guidelines for sharing of foundation seed stocks among states.

When the development of a new variety is the result of cooperative effort by a state or states and a federal agency, consideration for release should be a joint responsibility of the agencies involved. Appropriate use should be made of the services of National Variety Review Boards of the Association of Official Seed Certifying Agencies and the U.S. Plant Variety Protection Office in determining novelty of and in cataloging new varieties.

6. Standards for Release of Varieties

A variety should not be released unless it is distinctly superior to existing varieties in one or more characteristics important for the crop, or it is superior in overall performance in areas where adapted, and is at least satisfactory in other major requirements. A single major production hazard which a new variety can overcome, e.g., a highly destructive disease, may become the overriding consideration in releasing a variety. Varieties with a very limited range in adaptation should not be released unless performance in that limited range is outstandingly superior, or the variety possesses important use values not otherwise available, including diversification of the germplasm base for a species.

7. Naming and Registering of Varieties

(a) *Designation*

A new variety should be given a permanent designation before it is released. The designation should be acceptable to the states participating in the release, but the originating station or agency has the final responsibility. Brevity in designation is desirable. When this designation is a name, one short word is preferable; two short words are, however, acceptable. Meaningful number designations or combinations of words, letters, and numbers, consistent with accepted procedures, are also acceptable.

The International Code of Nomenclature for Cultivated Plants provides guides for the naming of varieties. It is recommended that this source be consulted with respect to new variety names.

(b) *Use of names*

Under no circumstances should a variety be distributed under more than one name, nor should the same name be used more than once in a given crop. Similar names should also be avoided. Provisions of the Federal Seed Act (53 Stat. 1275) apply.

Once established, a legitimate varietal name should not be changed. Names which are misleading or which are identical or similar to brand names or trademarks associated with agricultural products should be avoided, as there may be an implied association of the variety and trade names or trademarks. Proposed names should be cleared for possible infringement of trademarks, and previous use of the proposed variety name. This can best be accomplished by contacting the Seed Branch, Grain Division, Agricultural Marketing Service.

(c) *Registering varieties*

Information on new varieties of crops for which national variety review boards have been established should be submitted to the review board following consideration by the state variety committee but before final release is made.

New varieties of crops should be registered. Information for the registration or listing of

varieties should be submitted promptly following registration of the variety with the Crops Science Society of America or the listing of the variety with the American Society for Horticultural Science. Procedures for the registration of varieties are available from CSSA, and procedures for listing of varieties are available from ASHA.

8. Definition of Seed Classes and Certification Standards

The Association of Official Seed Certifying Agencies in its "Certification Handbook," Publication No. 23, dated June 1971, defines the various classes of seed and certification standards. These definitions as they now stand and as they may be amended in the future are hereby made a part of this policy.

9. Increase and Maintenance of Breeder Seed

(a) *Responsibility for maintaining breeder seed*

The originating Station or Agency should prepare a statement of plans and procedures for maintenance of stock seed classes, including limitations on the number of generations through which the variety may be sold by variety name.

When a variety is sufficiently promising to justify consideration for release, breeder seed should be increased to the volume needed to produce and maintain required foundation seed. So long as a variety is retained on the recommended list of the originating state, that state should maintain a reasonable reserve of breeder seed, which will be used to replenish and restore foundation seed of the variety to the desired level of genetic purity. When the variety is distributed in several states, or when the originating state or agency ceases to maintain breeder seed of a variety, a mutually satisfactory plan should be formulated by the interested states or agencies regarding the maintenance of breeder seed. Interested states should be notified well in advance by the originating state or agency when it plans to discontinue maintenance of breeder seed of a variety.

When a variety is to be released jointly by two or more states, a procedure should be formulated for a supply of breeders seed to be made available to each state.

(b) *Supplying sample of seed to National Seed Storage Laboratory*

A sample of breeder or foundation seed of all newly released varieties should be supplied by the originating state or agency to the National Seed Storage Laboratory, Fort Collins, Colorado. Recording forms are provided by that laboratory.

10. Increase, Maintenance, and Distribution of Foundation Seed

(a) *Multiplication of foundation seed*

An adequate and recurring supply of foundation seed is of prime importance in the multiplication of a variety. Reserves of foundation seed should be maintained to assure a continuing supply in the event of a seed crop failure. Foundation seed of publicly produced varieties should be increased under official guidance. It should be produced by those who have the experience, facilities, and skill to assure adequate supplies of seed with acceptable levels of genetic purity.

(b) *Distribution of foundation seed*

Minimal problems arise when there is simultaneous release of foundation seed of a new variety in all interested states. When a variety release is not simultaneous, distribution of foundation seed may present problems among the states. When foundation seed is distributed into another state where the variety is being distributed under allocation as a new release, the foundation seed should be offered through, or with the concurrence of, the official seed stocks or certifying agency in that state.

(c) *Basic principles in foundation seed programs*

Foundation seed should be released in a manner that will be of the greatest benefit to users and the public in general. Foundation seed should not be used for speculative purposes. Within this context, Foundation Seed programs should recognize the following basic principles:

(1) Qualified seed growers and seedsman should have an opportunity to obtain appropriate planting stocks of unrestricted varieties at an equitable cost, recognizing that selective allocations may be necessary to achieve increases to meet the needs of potential users.

(2) Restricted release of breeder and/or foundation seed of a variety is acceptable in situations and to the extent that general release to seed growers and/or seedsmen will not provide adequate seed of the variety on a continuing basis. If a restricted release policy is chosen for release of a variety, state and federal agencies, as well as private breeders (through state seed associations, ASTA, NCCPB) should be appropriately notified and given an opportunity to respond or bid on that particular variety.

(3) Planting stocks of varieties developed cooperatively with the agencies of USDA ordinarily will be made available through or with the concurrence of the seed stocks or certifying agency of the cooperating state(s) at an equitable cost to qualified seed growers and seedsmen. In special circumstances, e.g., No. 2 above, consideration may be given to granting limited term exclusive rights.

For this purpose, consideration should be given to applying for certificates of variety protection under the Plant Variety Protection Act. Where the new variety was developed cooperatively, the certificate will normally be assigned jointly to the USDA and the cooperator. When the cooperator is a public institution, title may be left with the cooperator provided he follows the guidelines set forth in Federal Regulations as to licensing.

11. Preparation and Release of Information

(a) *Coordination of publicity among states and agencies*

Seed producers, distributors, and users should be informed as fully as possible, consistent with variety testing policies and procedures within each state, of the values and the adaptation of new varieties in comparison with other available varieties.

Pertinent information as to the basic facts of origin and characteristics, and data justifying the increase and release of a new variety, shall be prepared by the fostering state(s) and or agency(ies) and provided to other interested states or agencies. The information used in deciding upon release of a new variety should also be used to inform seed producers, distributors, and the public of its value. Participating states or agencies should use this material, supported or modified by their own information, in state or national publicity. Publicity intended for national or regional periodicals should include information on the regional adaptation of the variety. A uniform date for the release of initial publicity should be agreed upon by the fostering states and/or federal agencies.

Appropriate information concerning actions with respect to Plant Variety Protection, including certification requirements, should be included in publicity releases, when appropriate.

The above procedure is intended to provide information that is complete, fair, and unbiased, and will make it possible for seed producers, distributors, and users to make sound judgments in selecting varieties.

(b) *Matching seed production and demand of varieties*

Seed production and demand must be developed together insofar as possible to assure that a variety will make its maximum contribution to agriculture. Thus, promotional publicity in advance of the release of a new variety, or before seed is available, or incomplete publicity following its release is not desirable. An educational program setting forth the superior characteristics, region of adaptation, and any special limitations which have been identified should be coordinated with seed supply.

APPENDIX C

Outline of Procedures for Seed Release of New Crop Varieties, Hybrids, or Genetic Stocks; Iowa Agriculture and Home Economics Experiment Station*

1. The research project leader for the crop involved establishes the merits of the selection through evaluations over a period of years. This usually involves state and regional nursery testing, but the regional aspects of testing may not always be accomplished or necessary, particularly where genetic stocks or inbred parental lines are involved. The guidelines and policies of the North Central Regional Committee (NCS-1) and of the Experiment Station Committee on Organization and Policy (ESCOP) setting forth principles to be followed by SEA-AR, United States Department of Agriculture, and experiment stations relative to development, multiplication, and distribution of publicly developed varieties also should be considered.
   a. The nature and extent of cooperative regional nursery testing with SEA-AR, the Soil Conservation Service, or other USDA agencies varies with individual crops, but standards of evaluation acceptable to the cooperating state and federal agencies for a specific crop should be met.
   b. Superiority for a new selection over varieties or hybrids currently available in at least one significant characteristic, or a complex of characteristics, should be firmly established

*February 1980. Prepared by R. E. Atkins, Agronomy, based on procedure now followed and suggestions of the Crop Breeding and Improvement Research Panel

before a proposal for release is considered. Three years of data are preferred when improved yield is the new character.

c. One year before a new selection is recommended to the departmental committee, a notification of tentative intent to release should be prepared by the project leader and sent to his department head and to his project (crop) counterpart in other states where the new variety may be expected to be produced. An offer to provide limited quantities of seed for testing may be included with the notification. This notification may not be necessary or appropriate for specific genetic stocks or inbred parental lines.

2. The research project leader prepares and presents the performance data and supporting information necessary for making a formal request to the Department's Variety Release Committee. Supporting information should include origin, pedigree, description of varietal characteristics, suggested name(s) or number for the new variety, justification for release, and proposed schedule for foundation seed increase and release of publicity.

3. If the proposal to release receives approval by the department evaluation committee, the request and information are submitted to the Department Head for approval and transmittal to the Associate Director of the Iowa Agriculture Experiment Station. Forms to accompany this request are available in the department office.

4. The Associate Director of the Iowa Agriculture Experiment Station appoints an *ad hoc* committee within the experiment station to consider the proposed release, and transmits the release and performance information to them for evaluation.

5. If release of the variety, hybrid, or genetic stock is recommended by the *ad hoc* committee of the Iowa Station and is approved by the Associate Director, the recommendation is transmitted to the Department Head and the notification of release to other states and to federal and commercial agencies continues as follows:

   a. When SEA-AR, SCS, or other USDA agency cooperation is involved directly in the development of the variety, the information and performance data relative to the release are transmitted by the Associate Director (in accordance with SEA-AR Administrative Memorandum 950.1 on preparation of joint release notices) to the Area Director of SEA-AR, and to the SCS or other USDA agency for their recommendation relative to joint release of the variety. Concurrence by the USDA is not mandatory for the originating state to proceed with a release of the variety.

   b. Investigation of prior use of the proposed variety name, possible conflicts with trademark or patent regulations, etc. will be conducted by the USDA personnel as well as by the project leader. In accordance with Article 15 of the International Code of Nomenclature for Cultivated Plants, variety names shall be markedly different from a scientific name of Latin form.

   c. For those crops where a variety review board is established, the project leader should obtain forms from the Chairman of the National Certified Variety Review Board and provide the information and performance data required by that board.

   d. Schedules and arrangements for the production of foundation seed are made cooperatively by the research project leader and the Production Manager, Committee for Agricultural Development, Iowa Agriculture Experiment Station and the increase of foundation seed is initiated.

   e. An offer to share foundation seed with other states is prepared cooperatively by the project leader and the Production Manager C.A.D., approved by the Department Head and Associate Director of the Iowa A.E.S., and sent to the appropriate counterparts in other states. Seed for increase should be offered to other states only one year in advance of the release of foundation seed to Iowa seed growers.

   Foundation seed allotment procedures vary with different crops. The procedure for release for each crop is approved by an Advisory Committee of the Committee for Agricultural Development. Different advisory committees are appointed for each crop. The small grain and soybean committee operates within the framework of an established

release policy approved by the Board of Trustees of the C.A.D. A general offer to share seed may not be appropriate or feasible for specific genetic stocks or inbred parental lines.

f. If protection under the provisions of the Plant Variety Protection Act of 1970 is recommended by the experiment station policy for the type of crop and seed, the research project leader for the crop involved shall obtain the forms and procedural information from the Plant Variety Protection Office that are necessary currently to secure the variety protection. The project leader provides the descriptive information requested, and submits the completed form(s) to the Associate Director of the Iowa A.E.S. for approval and transmittal to the Plant Variety Protection Office.

g. When USDA project cooperation is involved directly in the development and release of the variety, the release of information is accomplished in cooperation with the Assistant Administrator, Plant and Entomological Sciences SEA-AR, or the SCS, or other USDA agency. This agency will send appropriate letters of transmittal to the Experiment Station Director(s) of the state(s) involved for their concurrence.

h. If USDA projects or personnel are not directly involved, the preparation and dissemination of release information initiated by the project leader proceeds singularly and directly through the appropriate personnel of the Department, the Iowa Agriculture Experiment Station, the Committee for Agricultural Development, and the Information Service of Iowa State University.

i. A time for simultaneous news release relative to the new variety is established with cooperating states or federal agencies regardless of which of the two release alternatives (g, h) is implemented. To insure wide dissemination of the release information pertaining to new varieties, hybrids, or genetic stocks, a copy of the information released by the Director of the Experiment Station shall be sent to the Executive Secretary, National Council of Commercial Plant Breeders and to the Executive Secretary, American Seed Trade Association at the same time that it is disseminated to the state and federal agencies. For certain materials, such as genetic stocks of rather restrictive value, a selective information release may be made to appropriate agencies rather than a general news release.

6. If appropriate for the crop and type of germplasm or variety being released, the project leader prepares a varietal registration paper and sends it to the Chairman of the Subcommittee for the specific crop on the Crop Science Society of American Committee on Varietal Registration, or to a similar body in another appropriate society. Registration papers are commonly submitted for new varieties of the self-pollinated grain crops, forage grasses, and legumes; for genetic stocks; for parental lines of hybrids; and for synthetics. The initiation of varietal registration papers should be left to the discretion of the project leader.

7. Concurrent with submission of the varietal registration paper, a sample of seed of a newly released variety, parental inbred line, or genetic stock must be supplied to the National Seed Storage Laboratory, Fort Collins, Colorado, by the project leader. Even though a varietal registration paper is not prepared, the project leader should supply the sample of seed at the same time the variety is officially released.

New Variety Release Procedures—Project Leader

1. Prepares statement of intent to release a new variety and an offer of limited seed for testing. This statement should be disseminated one year before the proposal for release is initiated in Iowa. The statement should be sent to the project leader's department head and to his project (crop) counterparts in other states where the new variety may be expected to be produced. Notification may not be necessary or appropriate for genetic stocks or specific inbred parental lines.

2. Prepares proposal for release, together with performance data and other pertinent information about the selection, and transmits the information to the Department's Variety Release Committee via the Department Head.
3. If proposal to release is approved by the department committee, the project leader submits the release proposal and information, via his Department Head, to the Associate Director of the Iowa Agriculture Experiment Station.
4. If release of the variety is recommended by an *ad hoc* Variety Release Committee of the Iowa A.E.S. and by the Associate Director, the project leader proceeds as follows:
   a. If SEA-AR, SCS, or other USDA agency cooperation is involved directly in the development of the variety, the release proposal and information is transmitted by the Associate Director of the Iowa A.E.S. (in accordance with SEA-AR Administrative Memorandum 950.1 on preparation of joint release notices) to the Area Director of SEA-AR and to the SCS, or other USDA agency involved for their recommendation relative to joint release of the variety. The project leader and USDA personnel on the project will be informed of the USDA recommendation and may be called upon to amplify or supply additional information pertinent to a joint release.
   b. Investigates the prior use of the proposed variety name and possible conflicts with trademark or patent regulations.
   c. For crops where a review board is established, the project leader obtains forms from the Chairman of the National Certified Review Board and provides the information and performance data requested to the chairman of that board.
   d. Makes arrangements cooperatively with the Production Manager, Committee for Agricultural Development, Iowa A.E.S. for increase of foundation seed.
   e. Prepares and sends, cooperatively with the Production Manager, C.A.D., Iowa A.E.S., an offer to share foundation seed. This offer is sent to the project leader's counterparts in other states where the new variety may be expected to be produced. Seed for increase should be offered to other states only one year in advance of the release of foundation seed to Iowa seed growers.
   f. If variety protection is recommended by the experiment station policy for the type of crop and seed, the project leader obtains the current forms from the Plant Variety Protection Office, supplies the information requested, and submits the application to the Associate Director of the Iowa A.E.S. for approval and transmittal to the Plant Variety Protection Office.
   g. The project leader supplies information for publicity release through the appropriate personnel of the Department, the Iowa A.E.S., and the Information Service of Iowa State University. If SEA-AR, SCS, or other USDA agency cooperation is involved directly in the development and release of the variety, the project leader works either directly, or indirectly through USDA personnel on the project with the Associate Director of the Iowa A.E.S., in disseminating the release information to the USDA agency. Timing of the publicity release for a variety developed with direct USDA agency participation is determined in cooperation with the Assistant Administrator, Plant and Entomological Sciences, SEA-AR, or the SCS.
   h. If appropriate for the crop and type of germplasm or variety being released, the project leader prepares a varietal registration paper and sends it to the Chairman of the Subcommittee for the specific crop of the Crop Science Society of America Committee on Varietal Registration, or to a similar body in another appropriate society.
   i. Concurrent with submission of the varietal registration paper, the project leader sends a sample of seed of the newly released variety, parental inbred line, or genetic stock to the National Seed Storage Laboratory (NSSL), Fort Collins, Colorado. Even though a varietal registration paper is not prepared, a sample of the seed should be sent to the NSSL at the time the variety is officially released.

New Variety Release Procedures—Department Head

1. Receives proposal for variety release from research project leader, and transmits proposal to Department's Variety Release Committee for evaluation and recommendation.
2. If the Department Variety Release Committee recommends release of the variety, the Department Head obtains the release proposal and information from the project leader for the crop and sends, with his recommendation, to the Associate Director of the Iowa Agriculture Experiment Station.
3. Department Head receives the recommendation of the Associate Director (and the *ad hoc* Variety Release Committee of the Iowa A.E.S.) relative to release of the variety. If release is recommended, the Department Head requests the project leader to continue with the activities necessary for seed increase and release.
4. If a varietal registration paper is prepared, the Department Head receives the completed forms for submission to the appropriate journal, plus the manuscript for review. He completes the necessary processing for approval of publication and returns the manuscript and forms to the project leader.

New Variety Release Procedures—Associate Director

1. If a new variety release receives approval by a Department's Variety Release Committee and the Department Head, the Associate Director receives the release proposal.
2. The Associate Director appoints an *ad hoc* committee within the Experiment Station to evaluate the proposal and make a recommendation relative to release of the variety.
3. After receiving the recommendation of the *ad hoc* Review Committee, the Associate Director returns his recommendation relative to release to the Department Head. Concurrently, if SEA-AR, SCS, or other USDA agency cooperation is involved directly in the development of the variety, the Associate Director also transmits the performance data and supporting information together with his recommendation for release to the Assistant Administrator, Plant and Entomological Sciences, SEA-AR, or the SCS of the USDA for their recommendation relative to making a joint release of the variety.
4. If release is recommended, and if variety protection is requested, the Associate Director receives the forms to request protection from the project leader involved and submits them to the Plant Variety Protection Office for consideration.
5. Receives documents from the Department Head that are necessary for the release and distribution of seed, and for the dissemination and publicity, through the appropriate agencies of Iowa State University and the Agri. Expt. Stn. for his approval and signatures prior to their distribution.
6. If SEA-AR, SCS, or other USDA agency cooperation is involved directly in the development and release of the variety, the Associate Director receives, either from the project leader or USDA personnel associated with the project, the information for publicity release for transmittal to the Assistant Administrator, Plant and Entomological Sciences, SEA-AR, or the SCS of the USDA, in order that timing of the publicity release may be coordinated among the agencies involved.
7. If a varietal registration paper is prepared, the Associate Director receives, via the Department Head, the forms for submission to the appropriate journal, plus the manuscript for review. He completes the necessary processing and returns the materials to the project leader.

APPENDIX D

Request for Release and Distribution of Plant Variety

Iowa Agricultural Experiment Station

Approval is hereby requested for release and distribution of the following variety:

Soybean strain A74-302012

Identification (Name and/or Number)

A74-302012 is a strain number. The name Pella has been proposed for the variety.

Pedigree

A74-302012 is an F_4 plant selection from the cross L66L-137 × Calland. L66L-137 is a line developed at the Illinois Agricultural Experiment Station. Calland is a variety developed at the Indiana Agricultural Experiment Station.

Origin

A74-302012 was developed by Project 2118, Department of Agronomy, Iowa State University.

Description (Taxonomic)

A74-302012 has purple flowers, tawny pubescence, tan pods, dull seed coat luster, yellow seed coat, and black hilum.

Performance

The agronomic performance of A74-302012 is compared with five commercial varieties in the following tables. The data are not complete for all comparisons in Iowa because the varieties were not always grown in the same test.

Reasons for Distribution

A74-302012 has several attributes that should be useful to Iowa farmers. (1) Its maturity is earlier than public varieties currently available for southern Iowa such as Williams, Woodworth, Oakland, and Cumberland. The earlier maturity will be useful to farmers in the south-central tiers of counties that heretofore have had to choose between an early variety like Beeson or a later variety like Woodworth. (2) The strain has resistance to race 1 of phytophthora rot. Its yield has been superior to Oakland and Calland, the two public varieties with similar resistance. (3) The strain has tolerance to all known races of phytophthora rot that is superior to public varieties of similar maturity.

Proposed Methods of Distribution

There were 300 bushels of breeder seed of A74-302012 produced in Iowa during 1978. The seed will be distributed to interested states for production of foundation seed in 1979. The foundation seed will be distributed to certified growers in 1980 for production of registered seed that can be sold to farmers for planting in 1981.

Publicity release will be August 15, 1979.

Recommended

_____ _____

Head of Department Date

_____ _____

Chairman, Review Committee Date

Approved

_____ _____

Director, Agricultural Experiment Station Date

United States Patent

United States Patent [19]
Buck

[11] **Plant 4,653**
[45] **Feb. 17, 1981**

[54] **GERANIUM PLANT NAMED SUPER-WALTZTIME**

[75] Inventor: **Griffith J. Buck**, Ames, Iowa

[73] Assignee: **Iowa State University Research Foundation, Inc.**, Ames, Iowa

[21] Appl. No.: **112,114**

[22] Filed: **Jan. 14, 1980**

[51] **Int. Cl.**[3] **A01H 5/00**
[52] **U.S. Cl.** **Plt./68**
[58] **Field of Search** PLT./68

Primary Examiner—James R. Feyrer
Attorney, Agent, or Firm—Chas. W. Rummler

[57] **ABSTRACT**

A new cultivar of geranium plant distinguished by flowers that have a delicate pink coloration that intensifies under high temperature and light intensities, the very large size of its inflorescence clusters, and a continuous and abundant flowering habit throughout the year with adequate length of photoperiod. This new plant is more compact and free branching and, because of its greater number of stems and a consistent tendency to produce flowers on a 2-4 node cycle, has a greater flowering potential than its predecessors.

1 Drawing Figure

1

BACKGROUND OF THE NEW PLANT

My new geranium plant originated as a seedling of "Skylark" × "Waltztime", both unpatented, the cross having been made by me at Iowa State University at Ames, Iowa, in the course of breeding efforts carried on by me since 1956 with the object of developing improved geranium cultivars having better adaptability to the summer climates of the upper Midwest of the United States, including tolerance to the high summer night temperatures and humidity and a tolerance to *Botrytis cinerea* infection. This new plant was selected by me for propagation because of the color character of its inflorescence and its apparent fulfillment of my principal objectives and propagation under my direction through successive generations by means of cuttings at the Iowa State University horticultural greenhouses has shown that its distinctive and novel characteristics hold true from generation to generation and appear to be firmly fixed. Propagation of this new geranium plant for the commercial market is now being done by meristem culture and cuttings at Connellsville, Pa.

DESCRIPTION OF THE DRAWING

My new geranium plant is illustrated by the accompanying full color photograhic drawing which shows a potted plant in full bloom, the colors shown being as nearly true as it is reasonably possible to obtain by conventional commercial photographic procedures.

2

Form: Bush.
Height: 25 to 40 cm. (25 cm. as a 4 inch pot plant)
Growth: Compact and vigorous.
Habit: Erect and free-branching with sturdy, strong stems.
Foliage
Quantity—Abundant
Leaves—Size: Medium large, about 10 to 11 cm. long × 7.5 to 9.5 cm. wide. Shape: Round-cordate with several rounded lobes and serrated margins. Texture: Firm and slightly velvety to the touch. Color: Upper side—138A with central zone of 146B. Under side—138B.
Petioles.—Length: 9 to 14 cm.

THE BUD

Size: 14 to 16 mm. long and 9 to 11 mm. wide.
Shape: Pointed ovoid.
Opening: Buds open slowly.
Color
When sepals first divide—154D.
When petals begin to unfurl—154D suffused with 41C.
Sepals: At first, erect, and later curling back.
Shape—Long-pointed ovate.
Color—Inside: 146D. Outside: 146C veined with 180A.
Calyx
Aspect.—Hairy.

DESCRIPTION OF THE NEW PLANT

The following is a detailed description of my new geranium cultivar with color designations according to The R.H.S. Colour Chart published by The Royal Horticultural Society of London, England, the data having been obtained from observations of the plants of this new cultivar grown in the horticultural greenhouses of Iowa State University at Ames, Iowa.

THE PLANT

Origin: Seedling.

Parentage

Seed parent.—"Skylark" (unpatented).

Pollen parent.—"Waltztime" (unpatented).

Classification

Botanic.—Pelargonium · Hortorum, Bailey.

Commercial.—Garden geranium.

Plant 4,653

3

Florets.—Shape: When bloom first opens—Cup-shaped. The cup flattens to saucer-shape as bloom matures. Petals: Number: 9 to 14, including petaloids. Form: Obovate to spatulate. Arrangement: Slightly imbricated. Color: Spring: White lightly suffused and veined with RHS 41C and a suggestion of a basal blotch of RHS 33A. Summer-Fall: White ground suffused with RHS 41C and developing a margin of RHS 42A. 33A at base of petal and extending into upper half of petal. Winter: White suffused with RHS 41C with a basal blotch of RHS 33A. Reverse Side of Petals: White suffused with RHS 154D; tinted and veined with RHS 41C. Petaloids: Number: 1 to 5. Size: 1.5 to 2.0 cm. Color: Same as petals. Pedicel: Length: 2.0 to 3.1 cm. Strength: Good.

Effect of weather: None.

Persistence: Florets hang on and dry.

Disease resistance: Tolerant to *Botrytis cinerea* as observed in area where the disease is prevalent.

Lasting quality: 7 days.

REPRODUCTIVE ORGANS

Stamens:

Anthers.—5 to 8 in number. Length: 3 to 4 mm. Arrangement: Cylindrical.

Peduncle: Strong and erect.

Length.—15 to 22 cm.

Aspect.—Hairy.

Color.—146B.

THE FLOWER

Flowering habit: Continuous and abundant throughout the year with adequate length of photoperiod.

Inflorescence:

Type.—Umbel.

Size.—Very large.

Diameter.—10 to 13 cm.

Depth.—6 to 8 cm.

Shape.—Semi-globular.

Number of florets or buds.—50 to 60.

Peduncle.—Sturdy and upright. Length: 15 to 25 cm. Color: 138A.

4

Filaments.—Length: 5 to 6 mm. Color: White.

Pollen.—Color—Reddish-Brown.

Pistils: Number—1.

Styles.—8 to 9 mm. long.

Stigma.—Color—Red-Brown.

Fruit: Partially fertile.

Shape.—Ovoid with a long beak.

Color at maturity.—Brownish-Black.

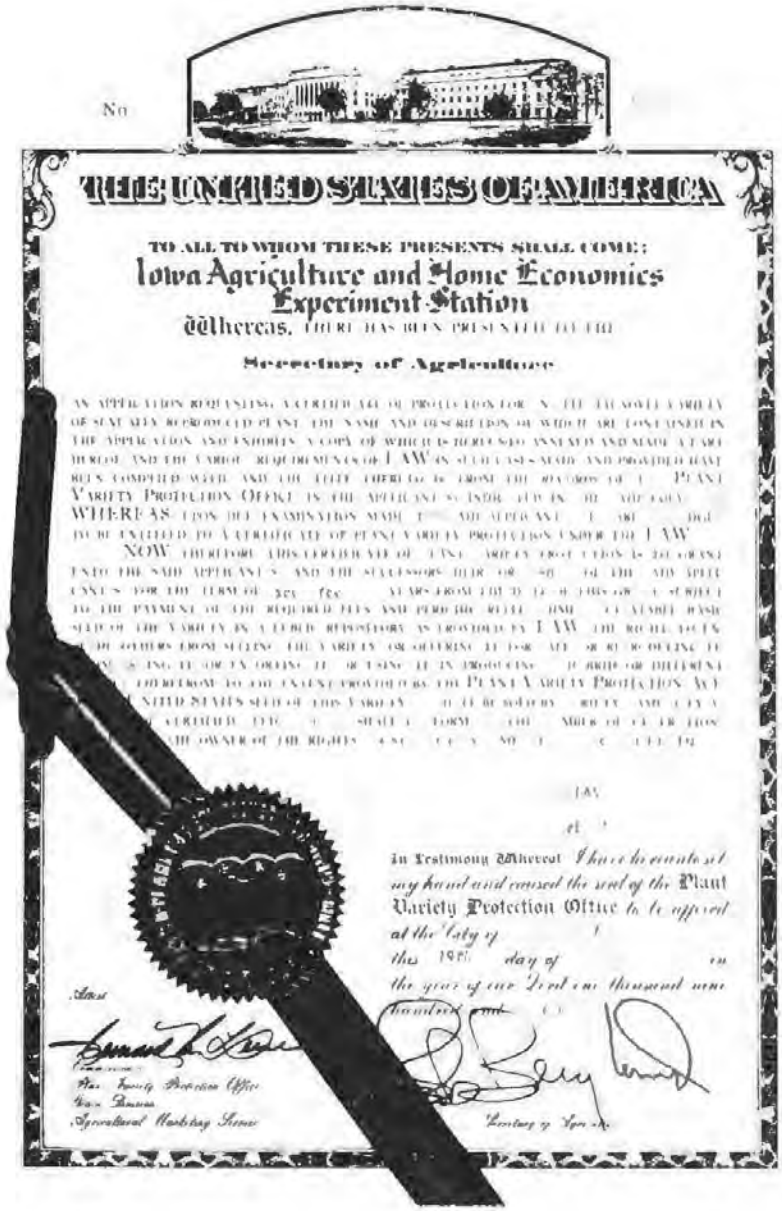
This geranium is similar in growth habit and cultural requirements to the older cultivars "Waltztime" and "Skylark". The flower color is in the same general color range of those cultivars but differs from them in that the color intensifies under high temperatures and high light intensities. This new plant is more compact and free branching, and because of its greater number of stems and a consistent tendency to produce flowers on a 2-4 node cycle, it has a greater flowering potential than the earlier cultivars.

I claim

1. A new and distinct cultivar of geranium plant substantially as herein shown and described, characterized by a vigorous and much branched growth habit and a continuous and abundant year around flowering under photoperiod culture.

* * * * *

Plant Variety Protection Certificate and Application



No

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Iowa Agriculture and Home Economics Experiment Station

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR A NEW AND DISTINCT VARIETY OF SOYBEAN REPRODUCED BY PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS A COPY OF WHICH IS HERETO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF THE PLANT VARIETY PROTECTION ACT RELATIVE THERETO HAVE BEEN COMPLIED WITH, AND THE FEES THEREFOR HAVE BEEN PAID TO THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT'S FAVOR, AS PROVIDED BY LAW;

WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT HAS COMPLIED WITH THE LAW AND APPEARS TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW;

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS ISSUED AND SEALED TO THE SAID APPLICANT, AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT, FOR THE TERM OF TWENTY-FIVE YEARS FROM THE TWENTY-FIRST DAY OF OCTOBER NINETEEN HUNDRED AND EIGHTY-THREE, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC RENEWAL FEES, AND CONVEYING THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT OR EXPORTING IT, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. IT IS UNLAWFUL TO SELL OR OFFER FOR SALE, OR REPRODUCE, OR IMPORT, OR EXPORT, OR USE IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, THE UNITED STATES SEED OF THIS VARIETY UNLESS AUTHORIZED BY THE OWNER OF THE RIGHTS UNDER THE PLANT VARIETY PROTECTION ACT. THIS CERTIFICATE DOES NOT EXTEND TO ANY INFRINGING USE OF THE VARIETY BY PERSONS OTHER THAN THE OWNER OF THE RIGHTS UNDER THE PLANT VARIETY PROTECTION ACT.

IN TESTIMONY WHEREOF, I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affixed at the City of this day of in the year of our Lord one thousand nine hundred and

Attest:

Plant Variety Protection Office
Dan Bauman
Agricultural Marketing Service

Secretary of Agriculture

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
LIVESTOCK, POULTRY, GRAIN & SEED DIVISION

FORM APPROVED
OMB NO. 40-R3822

No certificate for plant variety protection may be issued unless a completed application form has been received (5 U.S.C. 553).

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE

INSTRUCTIONS: *See Reverse*

1a TEMPORARY DESIGNATION OF VARIETY: A75-102032	1b VARIETY NAME: Weber	FOR OFFICIAL USE ONLY PV NUMBER	
2 KIND NAME: Soybean	3 GENUS AND SPECIES NAME: Glycine max.	FILING DATE	TIME A.M. P.M.
4 FAMILY NAME (BOTANICAL): Leguminosae	5 DATE OF DETERMINATION: August 31, 1979	FEE RECEIVED: $ ____ $ ____	DATE: ____ ____
6 NAME OF APPLICANT(S): Iowa Agriculture and Home Economics Experiment Sta.	7 ADDRESS (*Street and No. or R.F.D. No., City, State, and ZIP Code*): 104 Curtiss, Iowa State University, Ames, IA 50011		8 TELEPHONE AREA CODE AND NUMBER: 515-294-4762
9 IF THE NAMED APPLICANT IS NOT A PERSON, FORM OF ORGANIZATION (*Corporation, partnership, association, etc.*): State Experiment Station	10 IF INCORPORATED, GIVE STATE AND DATE OF INCORPORATION		11 DATE OF INCORPORATION

12 NAME AND MAILING ADDRESS OF APPLICANT REPRESENTATIVE(S), IF ANY, TO SERVE IN THIS APPLICATION AND RECEIVE ALL PAPERS: John P. Mahlstede, ISU - 104 Curtiss, Ames, IA 50011

13 CHECK BOX BELOW FOR EACH ATTACHMENT SUBMITTED:

- [X] 13A. Exhibit A, Origin and Breeding History of the Variety (*See Section 52 of the Plant Variety Protection Act.*)
- [X] 13B. Exhibit B, Novelty Statement.
- [X] 13C. Exhibit C, Objective Description of the Variety (*Request form from Plant Variety Protection Office.*)
- [ ] 13D. Exhibit D, Additional Description of the Variety.

14a DOES THE APPLICANT(S) SPECIFY THAT SEED OF THIS VARIETY BE SOLD BY VARIETY NAME ONLY AS A CLASS OF CERTIFIED SEED? (*See Section 83(a). If "Yes," answer 14B and 14C below.*) [X] YES [ ] NO

14b DOES THE APPLICANT(S) SPECIFY THAT THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS? [X] YES [ ] NO

14c IF "YES," TO 14B, HOW MANY GENERATIONS OF PRODUCTION BEYOND BREEDER SEED? [X] FOUNDATION [X] REGISTERED [X] CERTIFIED

15a DID THE APPLICANT(S) FILE FOR PROTECTION OF THIS VARIETY IN OTHER COUNTRIES? [ ] YES [X] NO (*If "Yes," give name of countries and dates.*)

15b HAVE RIGHTS BEEN GRANTED THIS VARIETY IN OTHER COUNTRIES? [ ] YES [X] NO (*If "Yes," give name of countries and dates.*)

16 DOES THE APPLICANT(S) AGREE TO THE PUBLICATION OF HIS/HER (THEIR) NAME(S) AND ADDRESS IN THE OFFICIAL JOURNAL? [X] YES [ ] NO

17 The applicant(s) declare(s) that a viable sample of basic seed of this variety will be furnished with the application and will be replenished upon request in accordance with such regulations as may be applicable.

The undersigned applicant(s) is (are) the owner(s) of this sexually reproduced novel plant variety, and believe(s) that the variety is distinct, uniform, and stable as required in Section 41, and is entitled to protection under the provisions of Section 42 of the Plant Variety Act.

Applicant(s) is (are) informed that false representation herein can jeopardize protection and result in penalties.

(DATE) _____ (SIGNATURE OF APPLICANT) _____

(DATE) _____ (SIGNATURE OF APPLICANT) _____

FORM GR-470 (1-8)

Exhibit A

Origin and Breeding History of the Variety. Weber is an F_5 plant selection from the cross C1453 × Swift. C1453 is a line developed by the USDA-SEA and Purdue University Agriculture Experiment Station from the cross C1266R × C1253. C1266R is a

selection from Harosoy × C1079, C1253 is from Blackhawk × Harosoy, and C1079 is from Lincoln × Ogden. F_5 seed was obtained by the Iowa Agriculture and Home Economics Experiment Station from Improved Variety Research, Inc. who made the cross and advanced it to the F_5 generation by single-seed descent in Iowa, Hawaii, and Puerto Rico. Progeny of F_5 plants were evaluated in 1973 for iron deficiency chlorosis on calcareous soil and the line had an adequate level of resistance. It was tested for yield in Iowa during 1974 and 1975, and in the Northern Regional Soybean Tests from 1976 to 1978 under the designation A75-102032.

Seed of Weber was increased in Iowa in 1978 and distributed to foundation seed organizations in states participating in its release. Foundation seed was produced in 1979. Foundation seed will be distributed to certified seed growers for planting in 1980.

The seed of Weber has met the purity standards for foundation seed. To meet this standard, a variety cannot have over 0.1% off-types or variants present.

Weber has shown evidence of stability. The attached data indicate a stable variety.

From Regional Summary of Uniform Test I

Strain	Yield (bu/A)	Rank No.	Maturity Date	Lodging Score	Height (in.)	Seed Quality Score	Seed Size (g/100)	Seed Composition	
								Protein (%)	Oil (%)
					1978				
No. of Tests	13	13	12	13	12	9	12	6	6
Coles	40.1	11	+6.2	2.2	38	2.1	19.5	42.3	20.2
Corsoy (II)	40.5	10	+7.8	2.0	37	2.4	16.3	41.4	20.5
Evans (0)	34.9	13	−6.3	1.2	30	2.9	16.2	40.3	22.2
Harlon	36.8	12	−5.2	1.6	34	2.5	17.4	39.6	21.8
Hodgson 78 (I)	41.7	5	9-20*	1.5	34	2.2	17.6	39.4	22.1
Weber	42.8	3	+4.2	1.8	36	2.3	13.7	40.2	21.4

*118 days after planting.

					1977–1978, 2-year mean				
No. of Tests	28	28	25	28	26	20	24	12	12
Coles	41.4	5	+6.9	2.3	39	2.1	19.2	41.1	20.2
Corsoy (II)	42.5	3	+7.4	2.2	38	2.3	16.1	40.0	20.6
Evans (0)	34.3	7	−7.8	1.4	31	2.5	15.6	39.2	22.2
Harlon	36.3	6	−5.6	1.8	35	2.4	16.8	38.7	21.8
Hodgson 78 (I)	41.5	4	9-17.5*	1.7	34	2.1	17.0	38.4	22.2
Weber	44.6	1	+4.8	2.0	36	2.1	14.0	39.0	21.4

*119 days after planting.

From Regional Summary of Uniform Test I

Cultivar	Yield (bu/A)	Maturity Date	Lodging Score*	Height (in.)	Chlorosis Score+	Seed Size (g/100)	Seed Content	
							Protein (%)	Oil (%)
Weber	44.6	Sept. 22	2.0	36	1.8	14.0	39.0	21.4
Hodgson 78	41.5	Sept. 18	1.7	34	2.0	17.0	38.4	22.2
Coles	41.4	Sept. 24	2.3	39	2.8	19.2	41.1	20.2
Corsoy	42.5	Sept. 25	2.2	38	3.6	16.1	40.0	20.6

*Scores range from 1 (plants erect) to 5 (plants prostrate).

+ Scores range from 1 (very good) to 5 (very poor).

Exhibit B

Novelty Statement. Weber most closely resembles Corsoy and Chippewa. Weber has white flowers, tawny pubescence, brown pods, a dull yellow seed coat and a black hilum. Corsoy has purple flowers, grey pubescence, brown pods, dull yellow seed coat and a yellow hilum. Chippewa has purple flowers, tawny pubescence, brown pods, shiny yellow seed coat and a black hilum. Weber has a seed size of 14.0 g/100 seeds, and Corsoy has 16.1 g/100 seeds.

Weber is 3 days earlier in maturity than Corsoy, and has a 5% higher yield. It has similar height and lodging scores as Corsoy. Weber is 1% lower in protein and 0.8% higher in oil and is 2.1 g/100 seeds smaller in size. Weber also has moderate resistance to iron chlorosis while Corsoy is susceptible.

Weber's unique combination of small seed size, chlorosis resistance and other traits listed on this form distinguish it from other varieties.

FORM GR-470-2
(8-15-72)

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
GRAIN DIVISION
HYATTSVILLE, MARYLAND 20782

EXHIBIT C
(Soybean)

OBJECTIVE DESCRIPTION OF VARIETY
SOYBEAN (*GLYCINE MAX*)

INSTRUCTIONS See Reverse.

NAME OF APPLICANT(S)
Iowa Agriculture and Home Economics Experiment Station

ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code)
104 Curtiss
Iowa State University
Ames, IA 50011

FOR OFFICIAL USE ONLY
PVPO NUMBER
VARIETY NAME OR TEMPORARY DESIGNATION

Place the appropriate number that describes the varietal character of this variety in the boxes below.

1. SEED SHAPE:
[1] 1 = SPHERICAL 2 = SPHERICAL FLATTENED 3 = ELONGATE 4 = OTHER (Specify)

2. SEED COAT COLOR:
[1] 1 = YELLOW 2 = GREEN 3 = BROWN 4 = BLACK 5 = OTHER (Specify) _____
SHADE: [2] 1 = LIGHT 2 = MEDIUM 3 = DARK

3. SEED COAT LUSTER
[1] 1 = DULL 2 = SHINY

4. SEED SIZE
[1][4] GRAMS PER 100 SEEDS

5. HILUM COLOR
[6] 1 = BUFF 2 = YELLOW 3 = BROWN 4 = GRAY 5 = IMPERFECT BLACK 6 = BLACK 7 = OTHER (Specify) _____
SHADE: [2] 1 = LIGHT 2 = MEDIUM 3 = DARK

6. COTYLEDON COLOR: [1] 1 = YELLOW 2 = GREEN

7. LEAFLET SIZE (See Reverse): [2] 1 = SMALL 2 = MEDIUM 3 = LARGE

8. LEAFLET SHAPE: [1] 1 = OVATE 2 = OBLONG 3 = LANCEOLATE 4 = ELLIPTICAL 5 = OTHER (Specify)

9. LEAF COLOR (See reverse): [1] 1 = LIGHT GREEN 2 = MEDIUM GREEN 3 = DARK GREEN

10. FLOWER COLOR: [1] 1 = WHITE 2 = PURPLE 3 = OTHER (Specify)

11. POD COLOR: [2] 1 = TAN 2 = BROWN 3 = BLACK

12. POD SET: [1] 1 = SCATTERED 2 = CONCENTRATED

13. PLANT PUBESCENCE COLOR: [2] 1 = GRAY 2 = BROWN 3 = OTHER (Specify)

SHADE: [2] 1 = LIGHT 2 = MEDIUM 3 = DARK

14. PLANT TYPES (See Reverse): [3] 1 = SLENDER 2 = BUSHY 3 = INTERMEDIATE

15. PLANT HABIT: [2] 1 = DETERMINATE 2 = INDETERMINATE 3 = OTHER (Specify)

16. HYPOCOTYL COLOR: [1] 1 = GREEN 2 = PURPLE

17. SEED PROTEIN: [-] 1 = A 2 = B

18. NUMBER OF DAYS TO FLOWERING (Place a zero in first box (e.g. [0][9]) when days are 9 or less.) [3][8] after emergence

19. MATURITY GROUP [3] 1 = 00 2 = 0 3 = I 4 = II 5 = III 6 = IV 7 = V 8 = VI 9 = VII 10 = VIII

20. SIZE OF 10 DAY OLD SEEDLING GROWN UNDER CONSTANT LIGHT (Growth Chamber) AT 75° C. (Place a zero in first box (e.g. [0][2]) when size is 9 mm. or less.)

[1][0][9] MM. LENGTH OF SEEDLING [1][8] MM. LENGTH OF COTYLEDON [1][1] MM. WIDTH OF COTYLEDON

21. DISEASE: (Enter 0 = Not Tested, 1 = Susceptible, 2 = Resistant)

[0] BACTERIAL PUSTULE	[0] SOYBEAN CYST	[0] DOWNY MILDEW	[2] PURPLE STAIN	[2] POD AND STEM BLIGHT	[0] ROOT KNOT
[1] PROGEYE	[0] STEM CANKER	[1] PHYTO-PHTHORA	[1] BROWN STEM ROT	[0] TARGET SPOT	[0] BROWN SPOT
[0] BUD BLIGHT	[0] WILDFIRE	[0] RHIZOCTONIA ROT	[2] OTHER (Specify) Iron chlorosis		

FORM GR-470-2 (REVERSE)

22. INDICATE WHICH VARIETY MOST CLOSELY RESEMBLES THAT SUBMITTED.

CHARACTER	NAME OF VARIETY	CHARACTER	NAME OF VARIETY
Plant shape	Corsoy	Petiole angle	-
Leaf shape	Hark	Seed size	Chippeva
Leaf color	Sloan	Seed shape	Rampage
Leaf surface	Corsoy	Seedling pigmentation	-

23. GIVE DATA FOR SUBMITTED AND SIMILAR STANDARD VARIETY

VARIETY	NO. OF DAYS TO MATURITY	LODGING SCORE	PLANT HEIGHT	LEAF SIZE		CONTENT		AVERAGE NO. OF PODS PER PLANT	IODINE NO.
				Width	Length	Protein	Oil		
Submitted Weber	124*	2.1	36	-	-	39%	21.4 %	-	-
Name of similar variety Corsoy	127*	2.3	38	-	-	40%	20.6 %	-	-

* after planting

INSTRUCTIONS

GENERAL The following publications may be used as a reference aid for completing this form:

1. Scott, Walter O. and Samuel R. Aldrich, 1970. Modern Soybean Production, The Farmer Quarterly.
2. Norman, A. G., 1963, The Soybean: Genetics, Breeding, Physiology, Nutrition, Management.
3. McKie, J. W., and K. L. Anderson, 1970, The Soybean Book.

LEAF COLOR Nickerson's or any recognized color fan may be used to determine the leaf color of the described variety. The following Soybean varieties may be used as a guide to identify the colors listed on the form.

COLOR	VARIETY
Light Green	"Ada"
Medium Green	"Wilkin"
Dark Green	"Swift"

LEAF SIZE The following varieties may be used as a guide to identify the relative size leaves.

SIZE	VARIETY
Small	"Amsoy"
Medium	"Bonus"
Large	"Anoka"

PLANT TYPE The following varieties may be used as a guide to identify the plant type.

TYPE	VARIETY
Slender	"Vansoy"
Intermediate	"Wirth"
Bushy	"Adelphia"

APPENDIX G

Facts About Naming and Labeling Varieties of Seed

Every year many new varieties of agricultural and vegetable seed reach America's marketplace. New seed varieties, when added to varieties already on the market, provide farmers and home gardeners with a wide selection of seed. But, in order for them to buy intelligently, seed must be correctly named and labeled. This is not always done.

Marketing a product by its correct name might seem to be the most likely way to do business. However, U.S. Department of Agriculture (USDA) seed officials have found that seed, unfortunately, is sometimes named, labeled, or advertised improperly as it passes through marketing channels.

Marketing seed under the wrong name is misrepresentation. It can lead to financial loss for several participants in the seed marketing chain.

The farmer, for example, buys seed to achieve specific objectives such as increased yield, competitiveness in a specialized market, or adaptability to growing conditions of a specific region. If seed is misrepresented and the farmer buys seed other than what was planned, the harvest may be less valuable than anticipated, or worse yet, there may not even be a market for the crop.

In one case, a farmer bought seed to grow cabbage to be marketed for processing into sauerkraut. As the cabbage matured, the farmer found that his crop was not suitable for processing and even worse, that he had no market for the cabbage in his fields. In this case financial hardship was brought about by improper variety labeling.

Seed companies and plant breeders also suffer in a market where problems with variety names exist. For instance, if the name of a newly released variety is misleading or confusing to the potential buyer, the variety may not attract the sales that it might otherwise.

This fact sheet outlines requirements for naming agricultural and vegetable seed. It is based on the Federal Seed Act, a truth-in-labeling law intended to protect farmers and home gardeners who purchase seed. Exceptions to the basic rules and the do's and don'ts of seed variety labeling and advertising also are explained.

Reprinted, by permission, from Agriculture Marketing Service, Naming and labeling varieties of seed, U.S. Department of Agriculture, AMS-592, Revised October 1981.

Who Names New Varieties?

The originator or discoverer of a new variety may give that variety a name. If the originator or discoverer can't or chooses not to name a variety, someone else may give that variety a name for marketing purposes. In such a case, the name first used when the seed is introduced into commerce will be the name of the variety.

It is illegal to change a variety name once the name has been legally assigned. In other words, a buyer may not purchase seed labeled as variety "X" and resell it as variety "Y." An exception to this rule occurs when the original name is determined to be illegal. In such an instance, the variety has to be renamed according to the rules mentioned above. Another exception to this rule applies to a number of varieties which were already being marketed under several names before 1956. (See section on synonyms.)

What's in a Name?

To fully understand what goes into naming a variety, you need to know the difference between a "kind" of seed and a seed "variety."

"Kind" is the term used for the seed of one or more related plants known by a common name such as carrot, radish, wheat, or soybean.

"Variety" is a subdivision of a kind. A variety has different characteristics from another variety of the same kind of seed. For example, "Oxheart" carrot and "Danvers 126" carrot or "Bragg" soybean and "Ransom" soybean.

The rules for naming plants relate to both kinds and varieties of seed:

1. A variety must be given a name that is unique to the kind of seed to which the variety belongs. For instance, there can only be one variety of wheat named "Prairie Road."
2. Varieties of two or more different kinds of seed may have the same name if the kinds are not closely related. For example, there could be a "Prairie Road" wheat and "Prairie Road" oat because wheat and oat are kinds of seed not closely related. On the other hand, it would not be permissible to have an "Alta" tall fescue and "Alta" red fescue because the two kinds of seed are closely related.
3. Once assigned to a variety, the name remains exclusive. Even if "Prairie Road" wheat has not been marketed for many years, a newly developed and different wheat variety can't be given the name "Prairie Road."
4. A company name may be used in a variety name as long as it is part of the original, legally assigned name. Once part of a legal variety name, the company name must be used by everyone including another company that might market the seed.

   When a company name is **not** part of the variety name, it should not be used in any way that gives the idea that it **is** part of the variety name. For example, Ajax Seed Company can't label or advertise "Prairie Road" wheat variety as "Ajax Prairie Road" since "Ajax" may be mistaken to be part of the variety name.

   The simplest way to avoid confusion is to separate the company and variety names in advertising or labeling.
5. Although USDA discourages it, you may use descriptive terms in variety names as long as such terms are not misleading. "GBR," for instance, is accepted among

sorghum growers as meaning "green bug resistant." It would be illegal to include "GBR" as part of a variety name if that variety were not green bug resistant. Similarly, if a sweet corn variety is named "Better Yield Bantam," the name would be illegal if this variety did not produce a higher yield than the standard Bantam sweet corn.

6. A variety name should be clearly different in spelling and in sound. "Alan" cucumber would not be permissible if an "Allen" cucumber were already on the market.

Hybrids

Remember that a hybrid also is a variety. Hybrid designations, whether they are names or numbers, also are variety names. Every rule discussed here applies to hybrid seed as well as to nonhybrid seed.

In the case of hybrids, however, the situation is potentially more complex since more than one seed producer or company might use identical parent lines in producing a hybrid variety. One company could then produce a hybrid that was the same as one already introduced by another firm.

When this happens, the same name must be used by both firms since they are marketing the same variety.

If the people who developed the parent lines have given the hybrid variety a name, that is the legal name. Otherwise, the proper name would be the one given by the company that first introduced the hybrid seed into commerce.

U.S. Department of Agriculture seed regulatory officials believe the following situation occurs far too often:

"State University" releases hybrid corn parent lines A and B.

John Doe Seed Company obtains seed of lines A and B, crosses the two lines, and is the first company to introduce the resulting hybrid into commerce under a variety name. John Doe Seed Company names this hybrid "JD 5259."

La Marque Seeds, Inc., obtains lines A and B, makes the same cross, and names the resulting hybrid variety "SML 25." There has been no change in the A and B lines that would result in a different variety. La Marque ships the hybrid seed, labeled "SML 25," in interstate commerce, and violates the Federal Seed Act because the seed should have been labeled "JD 5259."

Synonyms—Varieties with Several Names

As noted earlier, the name originally assigned to a variety is the name that must be used forever. It can't be changed unless it is illegal.

This does not mean that all varieties must be marketed under a single name. In fact, some old varieties may be marketed legally under more than one name. If several names for a single variety of an agricultural or vegetable seed were in broad general use before July 28, 1956, those names still may be used. For hybrid corn this exception applies to names in use before Oct. 20, 1951.

Here are some examples:

The names "Acorn," "Table Queen," and "Des Moines" have been known for many years to represent a single squash variety. They were in broad general use before July 28, 1956, so seed dealers may continue to use these names interchangeably.

If "Ajax 79EDX" hybrid field corn, released in 1949, also became known as "Golden Ajax 79EDX" in the late 1950s, it would be illegal to label or advertise that variety as "Golden Ajax 79EDX." If the two names had been in use before Oct. 20, 1951, the variety could then be marketed under either name.

With the exception of old varieties with allowable synonym names, all vegetable and agricultural varieties may have **only one** legally recognized name, and that name must be used by anyone who represents the variety name in labeling and advertising. This includes interstate seed shipments and seed advertisements sent in the mail or in interstate or foreign commerce.

Imported Seed

Seed imported into the United States can't be renamed if the original name of the seed is in the Roman alphabet.

For example, cabbage seed labeled "Fredrikshavn" and shipped to the United States from Denmark can't be given a different variety name such as "Bold Blue."

Seed increased from imported seed also can't be renamed. If "Fredrikshavn" were increased in the United States the resulting crop still couldn't be named "Bold Blue."

Seed with a name that is not in the Roman alphabet must be given a new name. In such a case, the rules for naming the variety are the same as stated previously.

Brand Names

USDA officials have found evidence of confusion over the use of variety names and brand or trademark names. This includes names registered with the Trademark Division of the U.S. Patent Office.

Here are some rules to keep in mind:

1. The brand or trademark name must be clearly identified as being other than part of the variety name. For example, "Red Giant Brand Arthur 71 wheat" adequately distinguishes between "Red Giant" brand and the variety "Arthur 71." "Red Giant Arthur 71 wheat," on the other hand, is not an adequate distinction.
2. A brand name must never take the place of a variety name.

   Let's say a firm uses "Super Nova" as a brand name for its line of sunflowers. This firm may not relabel or advertise variety "894" hybrid sunflower seed as variety "Super Nova" hybrid sunflower or even "Super Nova 894" variety.
3. If a brand or trademark name is part of a variety's name, the trademark loses status. Anyone marketing the variety under its name is required to use the exact, legal variety name, including brand or trademark.

   For instance, say Ajax Seed Company uses "Ajax Deluxe" as a brand or trademark for its line of vegetable seed. If the Ajax people introduce a new tomato variety named "Ajax Deluxe Cherry," they can't retain exclusive rights to that

name. If John Doe Seed Company later makes an interstate shipment of seed of this same variety, it must be labeled as "Ajax Deluxe Cherry."

Mixtures or Blends

The labeling and advertising of a varietal mixture or blend must not create the impression that the seed is a single variety.

The Federal Seed Act allows seed in mixtures or blends to be assigned a brand name but not a variety name. Either the percentages of each varietal component of the blend or the phrase "varieties not stated" must be printed on the label. This rule applies to 36 kinds of agricultural seed.

For example, if a soybean product were a blend of three varieties, the label or advertising could not read "Peninsula Soybean" because "Peninsula" could be mistaken for a variety name. The same soybeans could be sold as "Peninsula Brand Soybean Blend, Varieties Not Stated."

Vegetable seed containing more than one variety must be labeled with the name and percentage of each variety present. The "Varieties Not Stated" option can't be used.

Do Your Homework

If you are in a position to name a new variety, you should investigate the name you wish to use. You should not use a name if it has been used before, or if a confusingly similar name exists.

Let's say Ajax Seed Company is marketing a new variety of red clover called "Verdant." Unknown to Ajax, a "Verdant" red clover was released by another firm more than 18 years ago. This original "Verdant" never did become popular, and today it has all but disappeared from the marketplace. The fact that it has disappeared doesn't matter. Journals, old catalogs, or other records would prove the existence of the original "Verdant," and therefore Ajax Seed Company must rename its variety.

Researching a name to avoid potential conflict is not foolproof. The Seed Regulatory Branch in USDA's Agricultural Marketing Service can assist you in your research. However, there is no official registry of variety names, so the branch's files are incomplete. USDA can't assure you that a name is completely clear.

Summary

If the naming, labeling, and advertising of a seed variety is truthful, it is probably in compliance with the Federal Seed Act.

Keep these simple rules in mind to help eliminate violations and confusion in the marketing of seed:

- Research the proposed variety name before adopting it.
- Make sure the name cannot be confused with company names, brands, trademarks, or names of other varieties of the same kind of seed.

- Never change the variety name, whether marketing seed obtained from another source, or from your own production—for example, hybrid seed that already has a legal name.

For More Information

For more information on naming, labeling, and advertising seed, contact the Seed Regulatory Branch of the Livestock, Meat, Grain, and Seed Division, Agricultural Marketing Service, U.S. Department of Agriculture.

Write to: Seed Regulatory Branch, Rm. 2603-S, AMS, USDA, Washington, D.C., 20250, or call: (202) 447-9340.

The use of company and variety names in this guide is for illustration only and does not constitute endorsement or indictment by the U.S. Department of Agriculture.

Application for Review of Soybean Varieties for Certification

National Certified Soybean Variety Review Board

APPLICANT'S NAME Iowa Agric. & Home Econ. Expt. Station Date 1/18/80

ADDRESS Iowa State University, Ames, Iowa 50011

SPONSORING INSTITUTION (If other than applicant) _____

BREEDER'S NAME (If other than applicant) Walter R. Fehr

Variety Name Weber Experimental Designation A75-102032

The breeder, the sponsoring institution, or the organization must describe and DOCUMENT in this application those characteristics of the variety which give it distinctiveness by supplying the information requested below. Action will be deferred unless application is sufficiently documented. ANY STATED BENEFIT OR ADVANTAGES FOR THE VARIETY MUST BE SUPPORTED BY ACCEPTABLE DATA.

I. Indicate parentage (known variety or strain designation requested), breeding procedure (bulk, pedigree, etc.) and time sequence (generation) used in developing the variety. _____

II. VARIETY DESCRIPTION*

a. Maturity: 3 days earlier, or _____ days later than Corsoy
b. Flower Color: Purple _____, or White X
c. Pubescence Color: Brown X, or Gray _____.
d. Pod Color: Black _____, Brown X, or Tan _____.
e. Seedcoat Color: Yellow X, Black _____, Brown _____, Green _____ or other (describe) _____
f. Seed coat luster: Dull X, Shiny _____ or other _____
g. Hilum Color: Gray _____, Yellow _____, Black X, Brown _____, Imperfect Black _____, Buff _____, or other (describe) _____
h. Seed Shape: Round X, Elongate _____, or other (describe) _____
i. Seed Size: Seed per lb. 3200, as compared to Corsoy which has 2800 seeds per lb.
j. Plant height, inches: 36 compared to Corsoy at 38 inches.

*Compared with another widely grown variety of somewhat similar maturity grown in the area of usage and registered with the Crop Science Society of America. All comparisons should preferably be made to one variety.

(1)

k. Lodging score: 1 ____, 2 X, 3 ____, 4 ____, 5 ____ compared to Corsoy which was 1 ____, 2 X, 3 ____, 4 ____, or 5 ____. (Based on a score of 1 = erect to 5 = lodged flat.

l. Leaf size or shape: Large ____, Medium X, Small ____, Ovate X, Oval ____, Lanceolate ____, or other (describe) ____.

m. Plant Type: Slender ____, Bushy ____, or Intermediate X.

n. Plant Habit: Determinate ____, or Indeterminate X.

o. Other plant or seed characteristics that might help identify this variety (describe) ____

p. Protein content, if known: 39.0% compared to Corsoy with 40.0 %.

q. Oil content. 21.4% compared to Corsoy with 20.6 %.

III. DISEASE REACTION (List diseases and races for which rated and mark the reaction.)

Diseases (and races)	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible
a. Frog eye (Race 2)			X	
b. Brown Stem Rot			X	
c. Pod and Stem Blight		X		
d. Purple Stain		X		
e. Phytophthora (tolerance)				X
f. Phytophthora (race 1)				X
g. Chlorosis		X		
h.				

IV. INSECT REACTION (List insects and races for which rated and mark the reaction.)

Insects (and races)	Resistant	Moderately Resistant	No Resistance
a.			
b.			
c.			

V. (a) State any advantage(s) over varieties of similar maturity.

Weber has exhibited superior yield potential over Corsoy and other similar maturing varieties. On calcareous soils it has good resistance to iron deficiency chlorosis.

(b) State any distinguishing characteristics which demonstrate eligibility for certification. Weber has smaller seed size (14.0 g/100 Sd.) than Corsoy (16.1 g/100 Sd.). Weber is 1% lower in protein and .8% higher in oil.

(Note: at least one variety used for comparison shall be similar maturity and registered with the CSSA.)

VI. Performance Data (This is not mandatory, but would be of considerable value to the committee in deciding whether a variety is new and different or worthy of certification.) Yield and chemical data are required when either of these two traits is claimed as a distinctive characteristic. Note: Attach data when appropriate.

a. Performance data are X, are not ____ included with this application.

b. The data submitted are ____, are not X confidential to the committee.

VII. Tabular data concerning height, maturity, lodging, and seed size taken at or after maturity, is required. Yield and chemical composition is required only when either of these two traits is claimed as a distinctive characteristic. Data shall include appropriate check varieties registered with the CSSA. Data may be from tests conducted by private firms or Agricultural Experiment Stations, or both. Location(s) data collected All the locations growing Group I of the 1977-1978 Uniform Soybean Tests of Northern States.

PERFORMANCE DATA

Variety	No. of Tests	Height	Data Mature	Lodging Index	Seed Sz Wt./100 seeds	Oil Con-tent	% Pro-tein	Yield
See Attached Sheets								

VIII. List the geographic area or areas of adaptation of this variety Weber is of mid Group I maturity and best adapted to 43° to 44° N. Lat.

IX. State procedure for maintaining Foundation Seed, seed classes to be used, a statement as to limitations of generations that may be Certified, and any requirements or limitations necessary to maintain varietal characteristics. Seed of Weber was produced by foundation seed organizations in states participating in its release in 1979. Foundation seed will be distributed to certified seed growers for planting in 1980. This variety will be limited to 3 generations of production beyond breeder seed (ie. foundation, registered, and certified) that may be sold.

X. If this variety is accepted by Official certifying agencies, when will certified seed be offered for sale? Certified seed will be available in 1981.

XI. The Variety Review Board assumes all information on the application to be the responsibility of the originator or owner. If inaccuracies are later identified it will be the responsibility of the originator or owner to notify the Variety Review Board and to make corrections. When experimental designations are approved by the Variety Review Board and are later changed to a permanent name or number the originator or owner will notify the Variety Review Board and give them the new name and number.

XII. Is application for Plant Variety Protection contemplated? Yes X No ___ Undecided ___

At the time a variety is accepted for certification, a two pound sample seed lot of the generation, or generations, requested by the certifying agency shall be submitted to the agency by the sponsor. This lot(s) is to be retained as a control sample against which all future seed released for Certified Seed Production may be compared to establish continued trueness to variety.

XIII. The basis for determining eligibility for a Certificate of Plant Variety Protection by the PVP office is uniqueness or novelty. Descriptive data on all varieties whether protected or not is needed in determining such novelty. Please indicate below your wishes regarding such information from this application. Your decision will in no way affect deliberations of the Variety Review Board or the PVP office.

Descriptive information in this application may X, may not ___ by used by the PVP office in the varietal description data bank.

Submit twelve copies of this application and a one pound sample of the seed to:

Larry Svajar
Manager
Indiana Crop Improvement Association
3510 U. S. 52 South
Lafayette, Indiana 47905

If printed or mimeographed material is enclosed with this application, please send twelve copies of such matter.

Please submit a short and precise description of this variety in the space provided below, as you wish it published by AOSCA. Special emphasis on characteristics serving to identify this variety from others is requested.

Weber is a soybean variety of mid Group I maturity and best adapted to approximately 43° to 44° N. Lat. In comparison with Corsoy, Weber averages 5% higher in yield, 3 days earlier in maturity, similar in height and lodging resistance. Weber is 1% lower in protein and .8% higher in oil and 2.1 g/100 seeds smaller in size. Weber has white flowers, tawny pubescence, brown pods and dull yellow seeds with black hila. The white flowers and black hila distinguish Weber from Corsoy which has purple flowers and yellow hila.

OFFICIAL COMMITTEE ACTION

Approved_____ Deferred_____ Disapproved_____

Signed_____
Chairman, National Certified Soybean Variety Review Board

Date_____

Application for Review of Soybean Varieties for Certification

Weber is an F_5 plant selection from the cross C1453 × 'Swift.' C1453 is a line developed by the USDA-SEA and Purdue University Agricultural Experiment Station from the cross C1266R × C1253. C1266R is a selection from 'Harosoy' × C1079, C1253 is from 'Blackhawk' × 'Harosoy,' and C1079 is from 'Lincoln' × 'Ogden.'

F_5 seed was obtained by the Iowa Agriculture and Home Economics Experiment Station from Improved Variety Research, Inc., which made the cross and advanced it to the F_5 generation by single-seed descent in Iowa, Hawaii, and Puerto Rico. Progeny of F_5 plants were evaluated in Iowa in 1973 for iron deficiency chlorosis on calcareous soil, and the lines with adequate resistance were selected for yield evaluation. Weber was tested for yield in Iowa from 1974 to 1978, and in Northern Regional Soybean Tests from 1976 to 1978.

APPENDIX I

Questions Often Asked About Seed Certification

Questions often asked about certification of small grain and soybeans are listed below with brief answers. These interpretations of certification standards are supplemental to the certification requirements. The printed requirements take precedence over statements made herein.

A. The certifying agency

The Iowa Crop Improvement Association is designated by the Iowa Secretary of Agriculture as the official certifying agency in Iowa. It is a corporation composed of persons or concerns who are engaged in agricultural work in Iowa and are actively interested in crop improvement. It is *not* a government agency.

The Association establishes and administers requirements for certification and inspects the production of certified seed under those requirements.

The purposes of certification are to encourage production of: (1) high quality seed of superior varieties with high genetic purity, (2) to make known the sources of high quality seed, and (3) to encourage the use of good seed.

Varietal purity is the first consideration in seed certification but other factors such as germination and mechanical purity are also important. Fields and seed lots are rejected from certification when genetic standards are not met. Quality standards are listed as recommendations. Fields and seed lots are expected to meet quality standards but not required to meet them.

All applications for certification are made voluntarily.

B. Who can produce certified seed?

Anyone can produce certified seed provided: (1) the requirements for certification are followed, (2) there is an interest in learning how to produce certified seed, and (3) the

Courtesy of the Iowa Crop Improvement Association, Agronomy Building, Ames, Iowa, 50011

applicant is willing to spend the time and effort needed to; *remove off-type plants from production fields, control undesirable weeds and other crop mixtures, thoroughly clean planting and harvesting equipment before using*, and *thoroughly clean conveying and conditioning equipment and storage bins before using*.

C. What are the certification requirements?

Requirements for certification are updated annually and are available from the certification office. Briefly some of the more important requirements are listed below:

Plant an eligible variety.
Plant eligible seed.
Plant the seed on eligible land.

Eligible Varieties

Eligible varieties are those varieties that are accepted by the certifying agency as meriting certification in accordance with established eligibility requirements.

Eligible seed for planting

Genetic purity should be the first concern when purchasing seed for planting. A listing of certified seed growers is published each year and is available from the Association.

Foundation seed is eligible for planting and may be obtained from the Committee for Agricultural Development, Agronomy Building, Iowa State University, Ames, Iowa, or from the developer of a private variety. **Registered** seed is eligible for planting and may be purchased from other growers or seedsmen. **Certified** (i.e., the class of certified seed) is not eligible for planting to produce certified seed.

Eligible Land

The previous crop grown must be different from the one being produced. The land should be fertile to assure a reasonable yield of high quality seed. The land should also be relatively free of weeds.

D. What are the four classes of certified seed?

Breeder seed—This is seed which is in the hands of the developer or plant breeder.

Foundation seed originates from breeder seed. In Iowa it is produced by the Committee for Agricultural Development or the developer.

Registered seed must be grown from foundation, cleaned by the applicant or an approved seed conditioner, and bagged, when sampled and tagged and sealed when offered for sale. (Sealing is optional under certain conditions.)

Certified seed is usually grown from registered seed but may be grown from foundation seed. Certified seed must be cleaned by the applicant or an approved conditioner. Certified seed must be bagged, tagged, and sealed when sold (sealing is optional under certain conditions). *Exception*: Certified seed may be sold in bulk only by grower-applicants and/or Approved Seed Conditioners who sell directly to the consumer who will plant the seed. Bulk Retail Sales Certificates are to be used for bulk sales instead of blue certification tags.

E. What is meant by the term "limited generation"?

Limited generation is a system of controlling the number of generations of seed increased from foundation seed. The classes of seed of small grain and soybean recognized under the limited generation system are Foundation, Registered, and Certified. The certified class of seed is not eligible to be used as planting stock to produce additional generations of certified seed.

F. The steps of seed certification

There are three important steps in seed certification.

Production
Conditioning and Bagging
Merchandising

Growers considering seed certification for the first time should give careful consideration to each of these steps before planting time.

Rule of Thumb: **Do not attempt to produce certified seed unless it is known before planting how the seed will be conditioned and merchandised.**

Production. Growers who produce seed on their own land may find the production of certified seed the easiest step. Seedsmen who contract with others for the production of certified seed often find this a difficult step. A farmer grower working with a seedsman who specializes in conditioning and merchandising is often a good combination in making certified seed available to farmers.

Conditioning and Bagging Requires: adequate equipment (know equipment needs in advance), know-how (trained operators), time and labor, bulk and bag storage (clean, safe and easily accessible), and ability to keep records.

Merchandising Requires: knowledge of the market, sales ability, facilities for storage and delivery, financing, a system of bookkeeping, billing and collecting, and advertising and commissions.

Often arrangements can be made with Approved Conditioners to condition and merchandise certified seed.

G. What does it cost to certify seed?

Certification fees for *soybeans and small grains* are $20.00 for the first acre and $1.10 for each additional acre. Certification tags are $.04 each.

There will be additional expenses for: (1) seed for planting, (2) removal of off-type plants, other crops and noxious weeds, (3) cleaning of planting, harvesting, conditioning, and conveying equipment, and storage facilities, (4) conditioning and bagging and (5) marketing.

The cost of producing, conditioning, and merchandising a bushel of certified soybean seed could vary from $1.50 to $3.00 or more.

H. How to apply for certification

You will need to obtain a copy of the current Certification Requirements which are available from the Association by April 15 each year.

You will need to also request the forms necessary to make application—the forms will vary from crop to crop.

You will then need to complete the forms and send them to the Association along with your certification fee and a certification tag taken from the seed bag container of each lot of seed for each field you plant.

I. Inspections and sampling procedures

A representative of the Association will inspect each field before harvesting. For small grains this would be anytime after planting. For soybeans this is usually after most or all of the leaves have fallen from the plant. *Fields harvested before inspection are not eligible for certification.*

A representative of the Association will sample the seed after a request for sampling has been received in the Association office. The sample will be checked for varietal purity and

identity, analyzed for mechanical purity (pure seed, weed seed, other crop seed, and inert material), and tested for germination.

J. Certification tags and seals

If all requirements have been met, certification tags will be issued. The certification tags will have the following information printed on them:

Variety and Crop
Lot Number
Grower Number

To meet requirements of State and Federal Seed Laws a tag showing the analysis of the seed must also be attached to each container.

Approved Seed Conditioners may print and attach certification tags to seed after conditioning but before sampling. (See Certification Requirements for the procedure.)

Certification tags must be sewn or stapled to each bag of seed so they cannot be removed without damage to the tag.

Seals are required when the standards of another country, province, or state require sealing of the seed container. When seals are required, the responsibility for meeting this need will be assumed by the seller of the seed. Seals must be purchased from the Association and are available at cost plus shipping and handling.

K. What is an approved conditioner of certified seed?

Conditioners having necessary equipment, storage facilities and trained personnel may become Approved Conditioners of certified seed. Applications to become an Approved Conditioner of certified seed are required annually. The plant is inspected each year for a charge of $75.00 the first year applying and $60.00 for each succeeding year. The conditioning year begins July 1 and ends June 30.

An Approved Conditioner may be approved to condition one or more crops, which include small grain, soybeans, grasses, legumes, or corn.

An Approved Conditioner may be approved to; recondition, rebag, and retag certified seed under a specific procedure developed for this purpose, assume responsibility for completing certification of the original applicant following field inspection, and may print and attach certification tags to conditioned seed previous to sampling. (See Certification Requirements for procedure.)

L. Unanswered questions

If you have other questions about the certification of seed, send them to the Iowa Crop Improvement Association, Agronomy Building, Iowa State University, Ames, Iowa 50011 or call 515/294-6921 or see the current requirements for certification.

Glossary

A

additive gene effect Gene action in which the effects on a genetic trait are altered by each additional allele.

alien addition line Line with an extra chromosome or chromosome pair from another species.

alien substitution line Line in which a chromosome or chromosome pair from a donor species replaces a chromosome or chromosome pair of a recipient species.

A line Line with cytoplasmic male sterility and nonrestorer nuclear alleles that is used as the female parent in a cross to produce hybrid seed.

allele Alternative form of a gene at a locus.

allopolyploid Individual that has two or more genomes from different species.

amphidiploid Tetraploid whose somatic cells contain the diploid chromosome complements of two parental species.

anaphase Stage of meiosis or mitosis at which chromosomes of a homologous pair or chromatids of a chromosome separate and move toward opposite poles of a dividing cell.

androgenesis Development of a haploid embryo from a sperm nucleus after it enters the embryo sac.

aneuploid An individual with other than an exact multiple of the haploid chromosome complement due to the absence or presence of part or all of one or more chromosomes.

anther Pollen-bearing portion of the stamen.

anthesis Process of pollen shed from the anthers.

antibiosis Antagonistic association in which one organism has an injurious effect on normal growth and development of another.

apomixis Production of seed from an unfertilized egg or from somatic cells of the maternal plant.

asexual reproduction Reproductive process that does not involve the union of gametes, and which results in individuals with a genotype identical to the maternal parent.

asynapsis Lack of pairing between homologous chromosomes during meiosis.
autogamy Development of seed by self-pollination.
autopolyploid Individual that has more than two complete chromosome sets of a single genome.
avirulent Parasite unable to infect and cause disease in a host plant.

B

backcross Mating of a hybrid to one of its parents.
BC_1, BC_2, etc. Symbols used to designate the first backcross generation, the second backcross generation, and so forth.
bias Departure of a statistic from its true value.
bivalent Configuration consisting of two homologous chromosomes that pair during meiosis.
blend *See* Multiline.
***B* line** Male-fertile maintainer line with normal cytoplasm and nonrestorer nuclear alleles that is used to produce seed of its male-sterile *A*-line counterpart.
breeder seed Seed or vegetative propagules of a cultivar, generally produced by the breeder, from which all subsequent generations of multiplication of the cultivar originate.
breeding Genetic modification of living organisms.
bulk method Method of managing a segregating population during inbreeding that involves growing the population in a bulk plot, harvesting the self-pollinated seed of plants in bulk, and using a sample of the bulk to plant the next generation.

C

centromere Region of the chromosome to which the spindle fiber attaches during meiosis and mitosis.
certified seed (a) Seed of a cultivar that has been verified for its genetic identity and purity by an official seed certifying agency. Classes of certified seed are foundation, registered, and certified. (b) Class of certified seed that generally is produced from a planting of registered seed, but which also may be produced from foundation or certified seed.
character Expression of genes as revealed in the phenotype of a plant.
chasmogamy Pollination and fertilization occur in an open flower.
chiasma Position on the chromosome at which it is assumed that crossing over occurs during meiosis.
chromatid One of two identical sister strands of a replicated chromosome held together by a centromere.
chromosome Structural unit in the nucleus that carries genes in a linear order.
cleistogamy Pollination and fertilization occur in an unopened flower.
clone Individual that is reproduced asexually to produce progeny genetically identical to itself.
colchicine Alkaloid extracted from seeds or corms of *Colchicine autumnale* that induces doubling of the chromosome number of cells by interfering with spindle fiber formation and separation of daughter chromosomes during mitosis and meiosis.
combining ability (a) General combining ability is the average performance of the progeny of an individual when mated with a genetically diverse population or series of genotypes. (b) Specific combining ability is the performance

of the progeny from the mating of two specific genotypes in relation to the average performance of progenies from the mating of a series of genotypes in all combinations.

composite Mixture of genotypes from several sources that is maintained in bulk from one generation to the next.

correlation Statistical measure of the degree to which two characters vary together.

coupling linkage Linked recessive alleles occur in one homologous chromosome and the dominant alleles at the same loci occur in the other chromosome, such as *ab/AB*.

covariance Statistical measure of the interrelationship between two variables.

crossing over Exchange of segments between chromatids of homologous chromosomes during meiosis.

cross-pollination Female gametes of an individual are fertilized by the male gametes of other individual(s).

cultivar Cultivated variety of a plant.

cytoplasm Protoplasm of a cell excluding the nucleus.

cytoplasmic inheritance Transmission of hereditary factors through the cytoplasm instead of through genes in the nucleus.

D

deficiency Absence or deletion of a segment of a chromosome.

degrees of freedom Number of independent comparisons that can be made in a set of data.

deoxyribonucleic acid (DNA) Linear polymer in the chromosomes that carries the genetic information of an organism, and which consists of purine and pyrimidine bases linked through deoxyribose by phosphate groups.

detassel Removal of the tassel (male inflorescence) of maize.

diakinesis Stage of prophase I of meiosis at which chromosome contraction is near its maximum.

diallel mating Mating a group of genotypes in all possible combinations.

dihybrid Progeny of cross between parents that differ for the alleles at two loci.

dioecious Staminate and pistillate flowers occur on different plants of the same species.

diploid Individual with two sets of a basic genome; (2x).

diplonema Stage of prophase I of meiosis when homologous chromosomes repel each other and the chiasmata formed by crossing over between chromatids of homologous chromosomes are first clearly visible.

disjunction Separation of homologous chromosomes during the first meiotic division and of chromatids during anaphase of the second meiotic division and of mitosis.

dominance Intraallelic interaction at a heterozygous locus when one allele partially or completely masks the expression of another allele.

dominant gene effects Gene action with deviations from an additive condition, such that the heterozygote is more like one parent than the other.

donor parent Parent in a backcross from which one or more genes controlling a desired characteristic are transferred to the recurrent parent.

double cross Mating of two single-cross hybrids.

duplex Condition in which a polyploid has two dominant alleles at a locus, such as *AAaa* in a tetraploid. Condition at a locus in a tetraploid when the same

allele occurs on two homologous chromosomes and a second allele occurs on the other two homologous chromosomes, such as *aabb*.

duplication Repetition of a segment of a chromosome.

E

early-generation testing Method of managing segregating populations in which the genetic potential of heterozygous and/or heterogeneous individuals, lines, or populations are evaluated at an early stage of inbreeding.

egg The female gamete. In plants, the egg is one of the nuclei in the embryo sac of the pistil that is formed after mitotic division of a megaspore.

emasculation Removal of the anthers from a flower.

embryo Rudimentary plant in a seed.

embryo sac Female gametophyte that arises from the megaspore by successive mitotic divisions.

endosperm Triploid tissue of the seed that arises from fusion of a sperm nucleus with the two polar nuclei of the embryo sac.

environment External conditions that influence expression of genes of an individual.

epiphytotic Sudden and usually widespread development of a destructive disease.

epistasis Interallelic interactions between two or more loci that control the expression of a character.

euploidy Variations in chromosome number that are multiples of complete sets basic to a species.

evolutionary breeding Breeding procedure in which a cultivar is developed from a heterogeneous population that is subjected to natural selection over a number of generations.

experimental error Variation in the measurement of a genotype or treatment that results from unrecognized or uncontrolled factors in an experiment.

F

F_1 First filial generation or hybrid obtained from the mating of two genotypes. The F_2, F_3, and later generations represent successive generations of self-pollination.

family Group of individuals directly related to a common ancestor.

fertility Ability to produce viable offspring.

fertility restoring genes Nuclear genes that act to restore fertility in plants with male-sterile cytoplasm.

fertilization Fusion of an egg and sperm to form a zygote.

foundation seed Class of certified seed produced from breeder seed.

full-sib family Progeny of the mating of two individuals.

G

gamete Sex cell produced by the female and male reproductive organs.

gene Basic unit of inheritance which is a sequence of DNA nucleotides that codes for a functional product of RNA or a polypeptide.

gene deployment Planned geographic distribution of major genes for specific resistance to pests for use in cultivar development and production.

gene frequency Proportion in which alternative alleles of a gene occur in a population.

gene interaction Modification of gene action by a nonallelic gene or genes.

gene pool Genes available for the improvement of a species that are present within the species or are derived from other species.

general resistance Host plant resistance that functions against all races of a pest.

genetic drift Changes in gene frequency in small populations due to random processes.

genetic equilibrium Condition of a population in which successive generations contain the same genotypic and gene frequencies.

genetic marker Allele used to identify a gene, chromosome segment, or a chromosome.

genome Basic set of chromosomes of an organism.

genotype Genetic makeup of an individual.

genotypic ratio Proportions of different genotypes in a population.

germplasm Total of the genotypes that constitute a species.

H

half-sib family Progeny that have one parent in common.

haploid Cell or individual with half (n) of the somatic chromosome number.

heredity Transmission of genetic characters from parents to progeny.

heritability Portion of the phenotypic variation among individuals that is due to genetic differences among them. Broad-sense heritability is estimated from the ratio of the total genetic variance to the phenotypic variance. Narrow-sense heritability is estimated from the ratio of the additive portion of the genetic variance to the phenotypic variance.

heterogeneous population Condition that exists in a population of individuals with different genotypes.

heteromorphic flower Bisexual flower in which the stamens and style attain different lengths.

heterosis Condition in which a hybrid exceeds the performance of its parents for one or more characters. Mid-parent heterosis represents performance of the hybrid that exceeds the average performance of the parents per se. High-parent heterosis occurs when the hybrid performance exceeds that of the best parent.

heterozygous Different alleles are present at a locus on homologous chromosomes in a diploid or polyploid individual, such as *Aa* in a diploid and *AAaa* in a tetraploid.

hexaploid Individual with six sets of chromosomes; (6x).

homoeologous chromosomes Homologous or partially homologous chromosomes originating from different genomes.

homogeneous population Condition that exists in a population of individuals with the same genotype.

homologous chromosomes Chromosomes that pair during prophase of meiosis.

homomorphic flower Bisexual flower in which the stamens and style attain comparable lengths.

homozygous Identical alleles are present at a locus on homologous chromosomes in a diploid or polyploid individual, such as *AA* in a diploid or *AAAA* in a tetraploid.

horizontal resistance *See* General resistance.

hybrid Progeny of the mating between genetically different parents.

hybridization Mating of genetically different individuals.

hybrid vigor *See* Heterosis.

I

ideotype Ideal plant model formulated to assist in achieving selection goals.

imperfect flower Flower that does not possess both a stamen and a pistil.

inbred line Line produced by inbreeding that is homozygous and homogeneous.

inbreeding Mating of individuals that are related by descent.

inbreeding coefficient Quantitative measure of the degree of inbreeding, commonly denoted by the letter F.

inbreeding depression Reduction in performance that is associated with an increase in homozygosity.

incompatibility *See* Self-incompatibility.

independent assortment Random distribution of chromosomes to the gametes during meiosis.

inversion Rearrangement of a chromosome segment so that its genes are in reversed linear order.

irradiation Exposure of organisms to X-rays or other types of radiation to increase mutation rates or change chromosome structure.

isochromosome Chromosome with two identical arms.

isogenic lines (isolines) Lines that are genetically similar except for the alleles at one locus.

isolation Separation of a population of plants from other genotypes with which they are capable of mating.

L

landrace Cultivated forms that evolved from a natural population of a plant species.

leptonema Stage of prophase I of meiosis at which chromosomes have a thin, threadlike appearance and chromatids cannot be distinguished.

line Progeny of an individual plant.

linkage Condition in which genes located on the same chromosome are inherited together due to their close proximity.

linkage value Frequency of recombination between linked genes due to crossing over.

locus Position occupied by a gene on a chromosome.

M

M_1 First generation after treatment with a mutagenic agent. The M_2, M_3, and later generations represent successive generations of self-pollination.

male sterility Condition in which pollen is absent or nonfunctional in flowering plants. Genetic male sterility results from the action of nuclear genes. Cytoplasmic-genetic male sterility involves the interaction of cytoplasmic factors and nuclear genes.

mass-pedigree Management of a segregating population during inbreeding by use of the bulk (mass) method when conditions are unfavorable for selection, and use of progeny testing (pedigree) when conditions are favorable for selection.

mass selection System of breeding in which seeds from individuals selected on the basis of phenotype are bulked and used to grow the next generation.

mating system Method by which individuals are paired for crossing.

mean Arithmetic average of a series of observations.

megaspore One of four haploid spores originating from meiotic divisions of the megaspore mother cell in the ovary. One of the four megaspores undergoes mitotic division(s) to form the embryo sac.

meiosis Process that occurs in the female and male reproductive organs by which the chromosome number in somatic cells (2n) is reduced by one-half (n) in the haploid gametes.

metaphase Stage of mitosis or meiosis at which the chromosomes or homologous chromosome pairs are arranged in a linear manner at the center of the cell, immediately before the chromatids or homologous chromosomes separate and pass to the two poles during anaphase.

microspore One of the four haploid spores originating from the meiotic division of the microspore mother cell in the anther and which gives rise to the pollen grain.

mitosis Process by which the nucleus of a cell is divided into two genetically identical nuclei.

modified single cross Progeny of a mating between two related inbred lines and an unrelated inbred line.

modifying genes Minor genes that influence the expression of a major gene.

monoecious Staminate and pistillate flowers borne separately on the same plant.

monohybrid Cross between parents that differ by the alleles at one locus.

monoploid Individual that has the basic (x) chromosome number.

monosomic Individual lacking one member of a chromosome pair; 2n − 1 chromosomes.

multiline Seed mixture of isolines, closely related lines, or unrelated lines.

multiple alleles More than two alternative forms of a gene that can occur at a locus; e.g., *A*1, *A*2, *A*3, and *A*4.

multivalent Associations formed by the pairing of more than two homologous chromosomes during meiosis.

mutation Heritable variation in a gene or in chromosome structure.

N

nonpreference Mechanism for insect resistance in which plant characteristics make an individual undesirable to insects as a site for food, shelter, or reproduction.

nonrecurrent parent *See* Donor parent.

nulliplex Condition in which a polyploid has the same recessive allele at a locus on all homologous chromosomes. Condition at a locus in a polyploid in which only one allele, regardless of its dominance, is present on all homologous chromosomes such as *aaaa*.

nullisomic Individual that lacks both members of the same chromosome pair; 2n − 2 chromosomes.

O

open-pollination Natural cross-pollination.

outcross A cross, usually natural, to a plant with a different genotype.

ovary Enlarged basal portion of the pistil, in which the seeds are borne.

overdominance Condition in which the performance of the heterozygote for a gene exceeds that of the homozygotes.

P

pachynema Stage of prophase I of meiosis when the two chromatids of each chromosome may be distinguishable and homologous chromosomes have completely paired.

parthenogenesis Development of an embryo from a sex cell without fertilization.

pathogen Organism capable of inciting a disease.

pedigree Record of the ancestry of an individual.

pedigree method Selection procedure in a segregating population during inbreeding that involves maintenance of the ancestry of genotypes as selection is carried out among plants and their progeny each generation.

perfect flower Flower possessing both a stamen and pistil.

phenotype Appearance of an individual or group of individuals.

phenotypic ratio Proportions of different phenotypes in a heterogeneous population.

pistil Seed-bearing organ in the flower, composed of the ovary, style, and stigma.

pistillate flower Flower bearing a pistil but not stamens.

plant introduction Seeds or vegetative propagules of plants that have been introduced from another country.

polar nuclei Two nuclei in the embryo sac of the pistil that unite with a sperm nucleus in triple fusion. In some species, the cell formed by triple fusion is the origin of the endosperm.

pollen grain Male gametophyte originating from a microspore.

pollination Transfer of pollen from the anther to the stigma of the pistil.

polycross Open-pollination of a group of selected genotypes in isolation that have been arranged in a manner that promotes random mating.

polyploid Individual with more than two basic sets of chromosomes in its somatic cells; triploid (3x), tetraploid (4x), and hexaploid (6x).

population Community of individuals with a common origin.

probability Likelihood that an event will or will not occur.

progeny test Test of the genotypic value of an individual based on the performance of its offspring.

prophase Stage of meiosis or mitosis during which chromosomes coil and contract and during which homologous chromosomes synapse in meiosis.

protandry Maturation of the anthers before the pistil.

protogyny Maturation of the pistil before the anthers.

pseudogamy Absolute requirement for pollination to obtain viable apomictic seed, even though the sperm and egg nuclei do not unite.

pure line Homogeneous progeny of a homozygous individual.

pyramiding Incorporation of two or more major genes for specific resistance to a pest into a cultivar.

Q

quadriplex Condition in which a polyploid has four dominant alleles for a given locus, such as *AAAA* in a tetraploid.

qualitative character Character for which the phenotypic variation among genotypes is not continuous and can be separated into discrete classes.

quantitative character Character for which the phenotypic variation among genotypes is continuous and cannot be separated into discrete classes.

R

***R* line** Pollen parent line, containing fertility restoring alleles, that is crossed with an *A* line in the production of hybrid seed.

random Occurs by chance without any restrictions.

randomization Process of randomly arranging genotypes or other treatments in an experiment.

recessive Condition of an allele whose expression is masked in the presence of a dominant allele at the same locus on a homologous chromosome.

reciprocal cross Mating of two individuals in which each is used as the male parent in one cross and the female parent in the other.

recombination Formation of new combinations of genes as a result of the independent assortment that occurs in a heterozygous individual during meiosis.

recurrent parent Parent to which successive backcrosses are made in a backcross breeding program.

recurrent selection Cyclic selection in a breeding population to improve the frequency of desirable alleles for a character.

registered seed Class of certified seed generally produced from foundation seed, but which may also be produced from breeder seed.

regression Statistical measure of the rate of change in one variable as the level of the other variable is changed.

repulsion linkage Linkage between two heterozygous loci in which a dominant allele at one locus is on the same chromosome as a recessive allele at the other locus, such as *aB*/*Ab*.

resistant Characteristic of a plant that is capable of suppressing or retarding development of a pathogen or other injurious factor.

roguing Removal of individuals that do not conform to the standard of other members of a cultivar or population.

S

S Symbol used to denote generations of self-pollination. In this book, the S_0 and F_2 generations are considered genetically equivalent; therefore, the $S_1 = F_3$ and the $S_2 = F_4$.

sample Finite series of individuals or observations taken from a population.

segregation Separation of allelic pairs and their distribution to different cells during meiosis.

selection Identification of individuals or lines that are more desirable than others in a heterogeneous population.

selection differential Difference between the mean performance of genotypes selected from a population and the overall population mean.

self-fertility Capability of producing seed with self-pollination.

self-incompatibility Inability of male gametes to effect fertilization of female gametes from the same individual.

self-pollination Process by which male and female gametes from the same individual unite to produce seed.

semigamy Reproductive process in which the sperm nucleus enters the egg cell, but does not fuse with the egg nucleus.

sexual reproduction Reproduction that involves the fusion of male and female gametes.

sib mating Mating of individuals within the same family or line.

simplex Condition in which a polyploid has one dominant allele for a locus on homologous chromosomes, such as *Aaaa* for a tetraploid. Condition at a locus in a tetraploid when the same allele occurs on three homologous chromosomes and another allele occurs on the other chromosome, such as *aaab*.

single cross Mating between two genetically different parents.

single-seed descent Breeding procedure used during the inbreeding of a segregating population in which plants are advanced by single seeds from one generation to the next.

species Unit of taxonomic classification into which genera are divided. Groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups.

specific resistance Host plant is resistant to specific races of a pest.

sperm nuclei The male gamete. In plants, two sperm nuclei are formed by mitotic division of the generative nucleus of a microspore. One sperm nucleus unites with the egg cell to form the zygote that is the origin of the embryo, and the other unites with the two polar nuclei in triple fusion, which is the origin of the endosperm in some plant species.

stability, genotypic Reliability of performance of a genotype or group of genotypes across different environmental conditions.

stamen Pollen-bearing organ in the flower composed of an anther and a filament.

staminate flower Flower bearing stamens but not a pistil.

strain A group of similar and related individuals.

susceptible Characteristic of a host plant such that it is incapable of suppressing or retarding a pathogen or other injurious factor.

Syn Symbol used to designate generations of propagation of a synthetic population. The Syn 0 represents the parent clones or lines, Syn 1 is the progeny of the first intermating of the parents, Syn 2 is the open-pollinated progeny of the Syn 1 generation.

synapsis Pairing of homologous chromosomes during prophase I of meiosis.

synthetic Population produced by intermating selected genotypes and which is produced from one generation to the next by open-pollination.

T

telocentric chromosome Chromosome consisting of a single arm with a terminal centromere.

telophase Stage of meiosis or mitosis when the chromatids or chromosomes have reached the poles of the cell following anaphase and changes in the contraction of the chromatids and chromosomes occur.

testcross Mating used to evaluate the genotype of an individual.

tetragenic Condition at a locus in a tetraploid when four different alleles occur on homologous chromosomes, such as *abcd*.

tetraploid Individual with four basic (x) sets of chromosomes.

three-way cross Progeny of the mating of three genetically different parents.

tolerance Ability of plants to perform well in the presence of a destructive pathogen, insect, nematode, or environmental condition.

topcross Type of testcross that involves the mating of a series of individuals to a common parent to produce half-sib families for evaluation.

transgressive segregation Occurrence of one or more individuals in a population whose performance for a character falls outside the range of the parents of the population.

translocation Change in the position of a segment of a chromosome to another location in the same or a different chromosome.

trigenic Condition at a locus in a tetraploid when the same allele occurs on two homologous chromosomes and two different alleles occur on the other chromosomes, such as *aabc*.

triplex Condition in which a polyploid has three dominant alleles for a locus on homologous chromosomes, such as *AAAa* in a tetraploid.

triploid Individual with three basic (x) sets of chromosomes.

trisomic Individual that has the diploid chromosome complement and one chromosome of the genome in triplicate; 2n + 1 chromosomes.

U

univalent Unpaired chromosome in meiosis.

V

variance Statistical measure of the mean squared deviation of individual measurements from the overall mean of the measurements.

variation Differences among individuals due to differences in their genetic constitution, their response to the environment, genotype × environment interaction, and experimental error.

variety Subdivision of a species for taxonomic classification. Used interchangeably with the term cultivar to denote a group of individuals that are distinct genetically from other groups of individuals in the species.

vertical resistance *See* Specific resistance.

virulence Capacity of a pathogen to cause a disease.

Z

zygote Cell formed by the union of female and male gametes.

zygonema Stage of prophase I of meiosis when the threadlike chromosomes pair.

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