PRINCIPLES OF CULTIVAR DEVELOPMENT

VOLUME 1

Theory and Technique

Walter R. Fehr

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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

Contents

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,

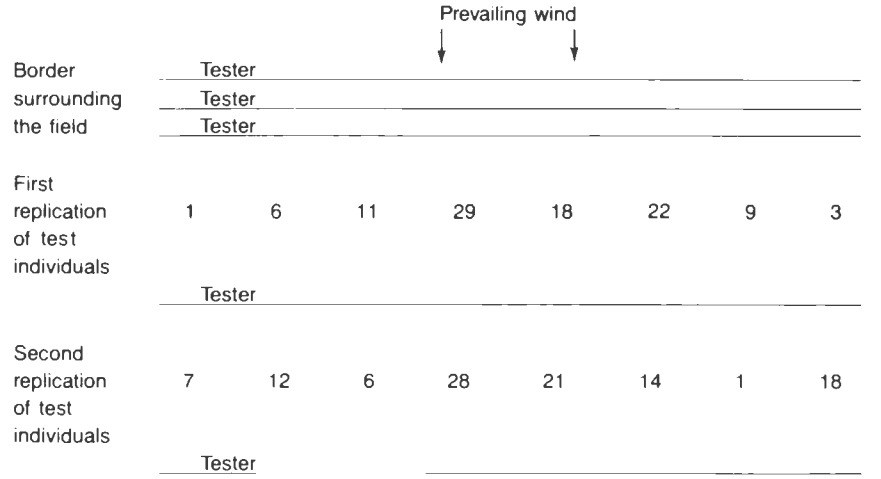


Figure 33-1 Layout of a nursery for production of testcross seed of clones to be evaluated for their combining ability.

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	$2^n - n + 1$

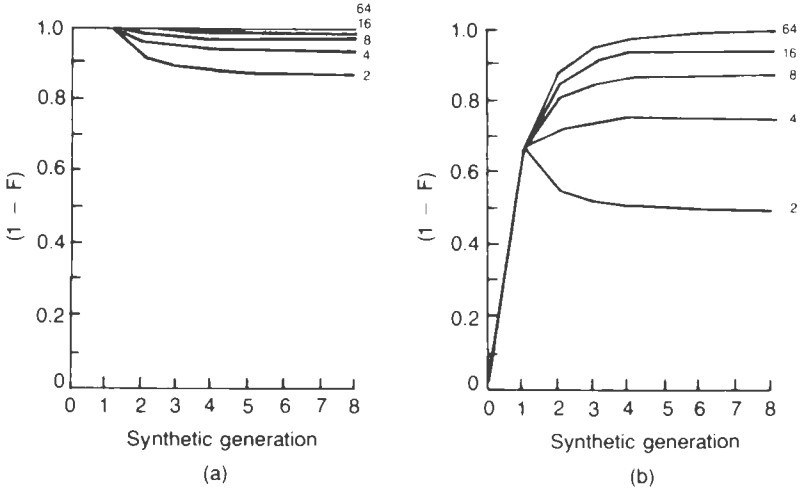
single crosses among n parents, and $\bar{P}$ is the mean performance of n parents. Random mating from the F_1 to F_2 generations and a lack of epistasis are necessary to obtain a good relationship between the predicted and empirical values for the F_2 generation. From the formula it is apparent that 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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