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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Crop Science, Vol. 8, 1968. M. K. Aycock, Jr., and C. P. Wilsie, Fig. 1, p. 484.

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Principles of Plant Breeding, © 1960. R. W. Allard, Table 14-1, p. 152. Reprinted by permission of John Wiley & Sons, Inc., New York.

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-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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