16

Breeding self-pollinated species

Purpose and expected outcomes

As previously discussed, self-pollinated species have a genetic structure that has implication in the choice of methods for their improvement. They are naturally inbred and hence inbreeding to fix genes is one of the goals of a breeding program for self-pollinated species in which variability is generated by crossing. However, crossing does not precede some breeding methods for self-pollinated species. The purpose of this chapter is to discuss specific methods of selection for improving self-pollinated species. After studying this chapter, the student should be able to discuss the characteristics, application, genetics, advantages, and disadvantages of the following methods of selection:

1 Mass selection.
2 Pure-line selection.
3 Pedigree selection.
4 Bulk population.
5 Single-seed descent.

And to:

6 Describe the technique/method of backcrossing.
7 Discuss the method of multiline breeding.
8 Discuss the method of breeding composites.
9 Discuss the method of recurrent selection.

Types of cultivars

At the beginning of each project, the breeder should decide on the type of cultivar to breed for release to producers. The breeding method used depends on the type of cultivar to be produced. There are six basic types of cultivars that plant breeders develop. These cultivars derive from four basic populations used in plant breeding – **inbred pure lines**, **open-pollinated populations**, **hybrids**, and **clones**. Plant breeders use a variety of methods and techniques to develop these cultivars.

Pure-line cultivars

Pure-line cultivars are developed for species that are highly self-pollinated. These cultivars are homogeneous and homozygous in genetic structure, a condition attained through a series of self-pollinations. These cultivars are often used as parents in the production of other kinds of cultivars. Pure-line cultivars have a narrow genetic base. They are desired in regions where uniformity of a product has a high premium.

Open-pollinated cultivars

Contrary to pure lines, **open-pollinated cultivars** are developed for species that are naturally cross-pollinated. The cultivars are genetically heterogeneous and heterozygous. Two basic types of open-pollinated cultivars are developed. One type is developed by improving the general population by **recurrent** (or repeated) **selection** or bulking and increasing material from selected superior inbred lines. The other type, called a **synthetic cultivar**, is derived from planned matings involving selected genotypes. Open-pollinated cultivars have a broad genetic base.

Hybrid cultivars

Hybrid cultivars are produced by crossing inbred lines that have been evaluated for their ability to produce hybrids with superior vigor over and above those of the parents used in the cross. Hybrid production exploits the phenomenon of hybrid vigor (or heterosis) to produce superior yields. Heterosis is usually less important in crosses involving self-pollinated species than in those involving cross-pollinated species. Hybrid cultivars are homogeneous but highly heterozygous. Pollination is highly controlled and restricted in hybrid breeding to only the designated pollen source. In the past, physical human intervention was required to enforce this strict pollination requirement, making hybrid seed expensive. However, with time, various techniques have been developed to capitalize on natural reproductive control systems (e.g., male sterility) to facilitate hybrid production. Hybrid production is more widespread in cross-pollinated species (e.g., corn, sorghum), because the natural reproductive mechanisms (e.g., cross-fertilization, cytoplasmic male sterility) are more readily economically exploitable than in self-pollinated species.

Clonal cultivars

Seeds are used to produce most commercial crop plants. However, a significant number of species are propagated by using plant parts other than seed (vegetative parts such as stems and roots). By using vegetative parts, the cultivar produced consists of plants with identical genotypes and is homogeneous. However, the cultivar is genetically highly heterozygous. Some plant species sexually reproduce but are propagated clonally (vegetatively) by choice. Such species are improved through hybridization, so that when hybrid vigor exists it can be fixed (i.e., the vigor is retained from one generation to another), and then the improved cultivar propagated asexually. In seed-propagated hybrids, hybrid vigor is highest in the F_1, but is reduced by 50% in each subsequent generation. In other words, whereas clonally propagated hybrid cultivars may be harvested and used for planting the next season's crop without adverse effects, producers of sexually reproducing species using hybrid seed must obtain a new supply of seed, as previously indicated.

Apomictic cultivars

Apomixis is the phenomenon of the production of seed without the benefit of the union of sperm and egg cells (i.e., without fertilization). The seed harvested is hence genetically identical to the mother plant (in much the same way as clonal cultivars). Hence, apomictic cultivars have the same benefits of clonally propagated ones, as previously discussed. In addition, they have the convenience of vegetative propagation through seed (versus propagation through cuttings or vegetative plant parts). Apomixis is common in perennial forage grasses.

Multilines

Multilines are developed for self-pollinating species. These cultivars consist of a mixture of specially developed genotypes called **isolines** (or **near isogenic lines**) because they differ only in a single gene (or a defined set of genes). Isolines are developed primarily for disease control, even though these cultivars could, potentially, be developed to address other environmental stresses. Isolines are developed by using the techniques of backcrossing in which the F_1 is repeatedly crossed to one of the parents (recurrent parent) that lacked the gene of interest (e.g., disease resistance).

Genetic structure of cultivars and its implications

The products of plant breeding that are released to farmers for use in production vary in genetic structure and consequently the phenotypic uniformity of the product. Furthermore, the nature of the product has implications in how it is maintained by the producers, regarding the next season's planting.

Homozygous and homogeneous cultivars

A cultivar may be genetically homozygous and hence produce a homogeneous phenotype or product.

Self-pollinated species are naturally inbred and tend to be homozygous. Breeding strategies in these species are geared toward producing cultivars that are homozygous. The products of economic importance are uniform. Furthermore, the farmer may save seed from the current season's crop (where legal and applicable) for planting the next season's crop, without loss of cultivar performance, regarding yield and product quality. This attribute is especially desirable to producers in many developing countries where the general tradition is to save seed from the current season for planting the next season. However, in developed economies with well-established commercial seed production systems, intellectual property rights prohibit the reuse of commercial seed for planting the next season's crop, thus requiring seasonal purchase of seed by the farmer from seed companies.

Heterozygous and homogeneous cultivars

The method of breeding of certain crops leaves the cultivar genetically heterozygous yet phenotypically homogeneous. One such method is hybrid cultivar production, a method widely used for the production of especially outcrossing species such as corn. The heterozygous genetic structure stems from the fact that a hybrid cultivar is the F_1 product of a cross of highly inbred (repeatedly selfed, homozygous) parents. Crossing such pure lines produces highly heterozygous F_1 plants. Because the F_1 is the final product released as a cultivar, all plants are uniformly heterozygous and hence homogeneous in appearance. However, the seed harvested from the F_1 cultivar is F_2 seed, consequently producing maximum heterozygosity and heterogeneity upon planting. The implication for the farmer is that the current season's seed cannot be saved for planting the next season's crop for obvious reasons. The farmer who grows hybrid cultivars must purchase fresh seed from the seed company for planting each season. Whereas this works well in developed economies, hybrids generally do not fit well into the farming systems of developing countries where farmers save seed from the current season for planting the next season's crop. Nonetheless, the use of hybrid seed is gradually infiltrating crop production in developing countries.

Heterozygous and heterogeneous cultivars

Other approaches of breeding produce heterozygous and homogeneous (relatively) cultivars, for example, synthetic and composite breeding. These approaches will allow the farmer to save seed for planting. Composite cultivars are suited to production in developing countries, while synthetic cultivars are common in forage production all over the world.

Homozygous and heterogeneous cultivars

Examples of such a breeding product are the mixed landrace types that are developed by producers. The component genotypes are homozygous, but there is such a large amount of diverse genotypes included that the overall cultivar is not uniform.

Clonal cultivar

Clones, by definition, produce offspring that are not only identical to each other but also to the parent. Clones may be very heterozygous but whatever advantage heterozygosity confers is locked in for as long as propagation is clonally conducted. The offspring of a clonal population are homogeneous. Once the genotype has been manipulated and altered in a desirable way, for example through sexual means (since some species are flowering, but are vegetatively propagated and not through seed), the changes are fixed for as long as clones are used for propagation. Flowering species such as cassava and sugarcane may be genetically improved through sex-based methods, and thereafter commercially clonally propagated.

Types of self-pollinated cultivars

In terms of genetic structure, there are two types of self-pollinated cultivars:

1. Those derived from a single plant.
2. Those derived from a mixture of plants.

Single-plant selection may or may not be preceded by a planned cross but often it is the case. Cultivars derived from single plants are homozygous and homogeneous. However, cultivars derived from plant mixtures may appear homogeneous but, because the individual plants have different genotypes, and because some outcrossing (albeit small) occurs in most selfing species, heterozygosity would arise later in the population. The methods of breeding self-pollinated species may be divided into two broad groups – those preceded by hybridization and those not preceded by hybridization.

Common plant breeding notations

Plant breeders use shorthand to facilitate the documentation of their breeding programs. Some symbols are standard genetic notations, while others were developed by breeders. Unfortunately there is no one comprehensive and universal system in use, making it necessary, especially with the breeding symbols, for the breeder to always provide some definitions to describe the specific steps in a breeding method employed in the breeding program.

Symbols for basic crosses

1 **F**. The symbol F (for **filial**) denotes the progeny of a cross between two parents. The subscript (x) represents the specific generation (F_x). If the parents are homozygous, the F_1 generation will be homogeneous. Crossing of two F_1 plants (or selfing an F_1) yields an F_2 plant ($F_1 \times F_1 = F_2$). Planting seed from the F_2 plants will yield an F_2 population, the most diverse generation following a cross, in which plant breeders often begin selection. Selfing F_2 plants produces F_3 plants, and so on. It should be noted that the seed is one generation ahead of the plant, that is, an F_2 plant bears F_3 seed.
2 ⊗. The symbol ⊗ is the notation for selfing.
3 **S**. The S notation is also used with numeric subscripts. In one usage $S_0 = F_1$; another system indicates $S_0 = F_2$.

Symbols for inbred lines

Inbred lines are described by two systems. System I describes an inbred line based on the generation of plants that are being currently grown. System II describes both the generation of the plant from which the line originated as well as the generation of plants being currently grown. Cases will be used to distinguish between the two systems.

Case 1. The base population is F_2. The breeder selects an F_2 plant from the population and plants the F_3 seeds in the next season.

System I: the planted seed produces an F_3 line.
System II: the planted seed produces an F_2 derived line in F_3 or an $F_{2:3}$ line.

If seed from the F_3 plants is harvested and bulked, and the breeder samples the F_4 seed in the next season, the symbolism will be as follows:

System I: the planted seed produces an F_4 line.
System II: the planted seed produces an F_2 derived line in F_4 or an $F_{2:4}$ line.

Case 2. The breeder harvests a single F_4 and plants F_5 seed in a row.

System I: the planted row produces an F_5 line.
System II: the planted row constitutes an F_4 derived line in F_5 or an $F_{4:5}$ line.

Similarly the S notation may be treated likewise. Taking case 1 for example:

System I: S_1 line.
System II: S_0 derived line in S_1 or an $S_{0:1}$ line.

Notation for pedigrees

Knowing the **pedigree** or ancestry of a cultivar enables the plant breeder to retrace the steps in a breeding program to reconstitute a cultivar. Plant breeders follow a short-hand system of notations to write plant pedigrees. Some pedigrees are simple, others are complex. Some of the common notations are as follows:

1 A slash, /, indicates a cross.
2 A figure between slashes, /2/, indicates the sequence or order of crossing. A /2/ is equivalent to // and indicates the second cross. Similarly, / is the first cross, and /// the third cross.
3 A backcross is indicated by *; *3 indicates the genotype was backcrossed three times to another genotype.

The following examples will be used to illustrate the concept.

Pedigree 1: MSU48-10/3/Pontiac/Laker/2/MS-64.
Interpretation:
(a) The first cross was Pontiac (as female) × Laker (as male).
(b) The second cross was [Pontiac/Laker (as female)] × MS-64 (as male).
(c) The third cross was MSU48-10 (as female) × [Pontiac/Laker//MS-64 (as male)].

Pedigree 2: MK2-57*3/SV-2.
Equivalent formula: MK2-57/3/MK2-57/2/MK2-57/SV-2.
Interpretation: the genotype MK2-57 was backcrossed three times to genotype SV-2.

Mass selection

Mass selection is an example of selection from a biologically variable population in which differences are genetic in origin. The Danish biologist, W. Johansen, is credited with developing the basis for mass selection in 1903. Mass selection is often described as the oldest method of breeding self-pollinated plant species. However, this by no means makes the procedure outdated. As an ancient art, farmers saved seed from desirable plants for planting the next season's crop, a practice that is still common in the agriculture of many developing countries. This method of selection is applicable to both self- and cross-pollinated species.

Key features

The purpose of mass selection is population improvement through increasing the gene frequencies of desirable genes. Selection is based on plant phenotype and one generation per cycle is needed. Mass selection is imposed once or multiple times (recurrent mass selection). The improvement is limited to the genetic variability that existed in the original populations (i.e., new variability is not generated during the breeding process). The goal in cultivar development by mass selection is to improve the average performance of the base population.

Applications

As a modern method of plant breeding, mass selection has several applications:

1. It may be used to maintain the purity of an existing cultivar that has become contaminated, or is segregating. The off-types are simply rogued out of the population, and the rest of the material bulked. Existing cultivars become contaminated over the years by natural processes (e.g., outcrossing, mutation) or by human error (e.g., inadvertent seed mixture during harvesting or processing stages of crop production).
2. It can also be used to develop a cultivar from a base population created by hybridization, using the procedure described next.
3. It may be used to preserve the identity of an established cultivar or soon-to-be-released new cultivar. The breeder selects several hundreds (200–300) of plants (or heads) and plants them in individual rows for comparison. Rows showing significant phenotypic differences from the other rows are discarded, while the remainder is bulked as breeder seed. Prior to bulking, sample plants or heads are taken from each row and kept for future use in reproducing the original cultivar.
4. When a new crop is introduced into a new production region, the breeder may adapt it to the new region by selecting for key factors needed for successful production (e.g., maturity). This, hence, becomes a way of improving the new cultivar for the new production region.
5. Mass selection can be used to breed horizontal (durable) disease resistance into a cultivar. The breeder applies low densities of disease inoculum (to stimulate moderate disease development) so that quantitative (minor gene effects) genetic effects (instead of major gene effects) can be assessed. This way, the cultivar is race-non-specific and moderately tolerant of disease. Further, crop yield is stable and the disease resistance is durable.
6. Some breeders use mass selection as part of their breeding program to rogue out undesirable plants, thereby reducing the materials advanced and saving time and reducing costs of breeding.

Procedure

Overview

The general procedure in mass selection is to rogue out off-types or plants with undesirable traits. This is called by some researchers, **negative mass selection**. The specific strategies for retaining representative individuals for the population vary according to species, traits of interest, or creativity of the breeder to find ways to facilitate the breeding program. Whereas rouging out and bulking appears to be the basic strategy of mass selection, some breeders may rather select and advance a large number of plants that are desirable and uniform for the trait(s) of interest (**positive mass selection**). Where applicable, single pods from each plant may be picked and bulked for planting. For cereal species, the heads may be picked and bulked.

Steps

The breeder plants the heterogeneous population in the field, and looks for off-types to remove and discard

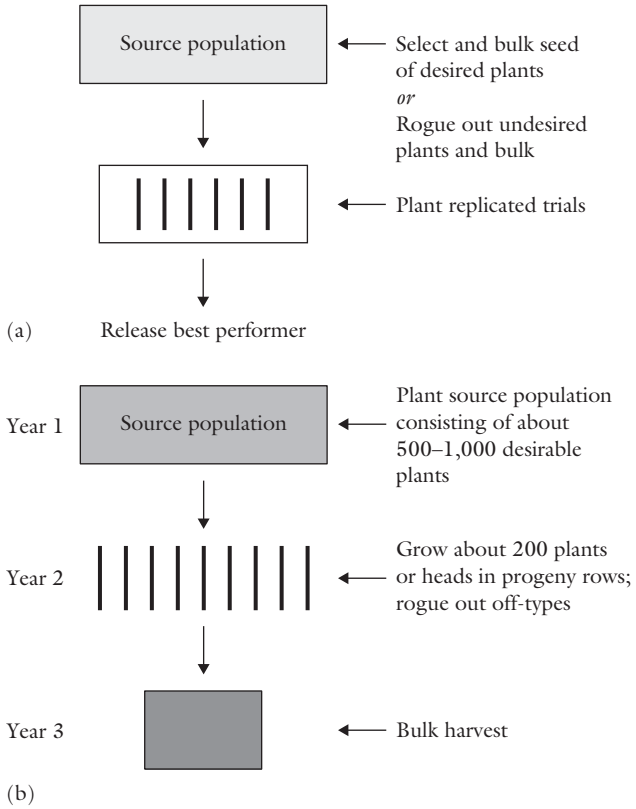

Figure 16.1 Generalized steps in breeding by mass selection for (a) cultivar development, and (b) purification of an existing cultivar.

(Figure 16.1). This way, the original genetic structure is retained as much as possible. A mechanical device (e.g., using a sieve to determine which size of grain would be advanced) may be used, or selection may be purely on visual basis according to the breeder's visual evaluation. Further, selection may be based on targeted traits (direct selection) or indirectly by selecting a trait correlated with the trait to be improved.

Year 1 If the objective is to purify an established cultivar, seed of selected plants may be progeny-rowed to confirm the purity of the selected plants prior to bulking. This would make a cycle of mass selection have a 2-year duration instead of 1 year. The original cultivar needs to be planted alongside for comparison.

Year 2 Evaluate composite seed in a replicated trial, using the original cultivar as a check. This test may be conducted at different locations and over several years. The seed is bulk harvested.

Genetic issues

Contamination from outcrossing may produce heterozygotes in the population. Unfortunately, where a dominance effect is involved in the expression of the trait, heterozygotes are indistinguishable from homozygous dominant individuals. Including heterozygotes in a naturally selfing population will provide material for future segregations to produce new off-types. Mass selection is most effective if the expression of the trait of interest is conditioned by additive gene action.

Mass selection may be conducted in self-pollinated populations as well as cross-pollinated populations, but with different genetic consequences. In self-pollinated populations, the persistence of inbreeding will alter population gene frequencies by reducing heterozygosity from one generation to the next. However, in cross-pollinated populations, gene frequencies are expected to remain unchanged unless the selection of plants was biased enough to change the frequency of alleles that control the trait of interest.

Mass selection is based on plant phenotype. Consequently, it is most effective if the trait of interest has high heritability. Also, cultivars developed by mass selection tend to be phenotypically uniform for qualitative (simply inherited) traits that are readily selectable in a breeding program. This uniformity not withstanding, the cultivar could retain significant variability for quantitative traits. It is helpful if the selection environment is uniform. This will ensure that genetically superior plants are distinguishable from mediocre plants. When selecting for disease resistance, the method is more effective if the pathogen is uniformly present throughout the field without "hot spots".

Some studies have shown correlated response to selection in secondary traits as a result of mass selection. Such a response may be attributed to linkage or pleiotropy.

Advantages and disadvantages

Some of the major advantages and disadvantages of mass selection for improving self-pollinated species are given here.

Advantages

1. It is rapid, simple, and straightforward. Large populations can be handled and one generation per cycle can be used.
2. It is inexpensive to conduct.

3 The cultivar is phenotypically fairly uniform even though it is a mixture of pure lines, hence making it genetically broad-based, adaptable, and stable.

Disadvantages

1 To be most effective, the traits of interest should have high heritability.
2 Because selection is based on phenotypic values, optimal selection is achieved if it is conducted in a uniform environment.
3 Phenotypic uniformity is less than in cultivars produced by pure-line selection.
4 With dominance, heterozygotes are indistinguishable from homozygous dominant genotypes. Without progeny testing, the selected heterozygotes will segregate in the next generation.

Modifications

Mass selection may be direct or indirect. Indirect selection will have high success if two traits result from pleiotropy or if the selected trait is a component of the trait targeted for improvement. For example, researchers improve seed protein or oil by selecting on the basis of density separation of the seed.

Pure-line selection

The theory of the pure line was developed in 1903 by the Danish botanist Johannsen. Studying seed weight of beans, he demonstrated that a mixed population of self-pollinated species could be sorted out into genetically pure lines. However, these lines were subsequently nonresponsive to selection within each of them (Figure 16.2). Selection is a passive process since it eliminates variation but does not create it. The pure-line theory may be summarized as follows:

1 Lines that are genetically different may be successfully isolated from within a population of mixed genetic types.
2 Any variation that occurs within a pure line is not heritable but due to environmental factors only. Consequently, as Johansen's bean study showed, further selection within the line is not effective.

Lines are important to many breeding efforts. They are used as cultivars or as parents in hybrid production (inbred lines). Also, lines are used in the development of genetic stock (containing specific genes such as disease resistance or nutritional quality) and synthetic and multiline cultivars.

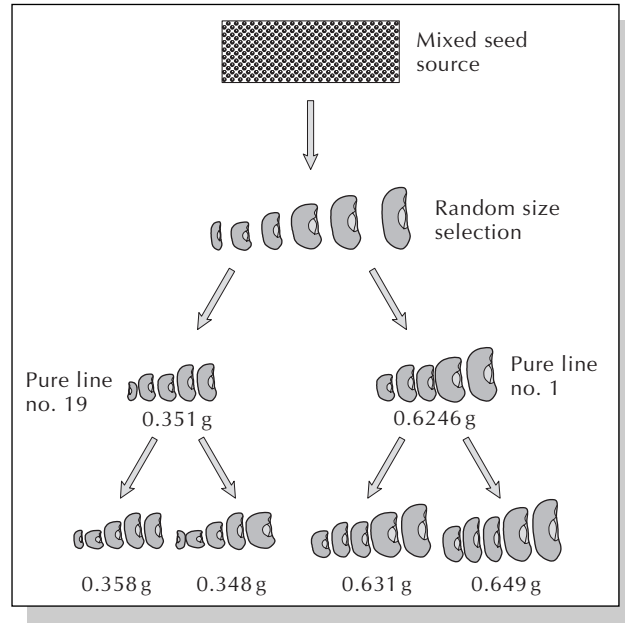

Figure 16.2 The development of the pure-line theory by Johannsen.

Key features

A **line cultivar**, by definition, is one that has a coefficient of parentage of at least 0.87. A **pure line** suggests that a cultivar has identical alleles at all loci. Even though plant breeders may make this assumption, it is one that is not practical to achieve in a breeding program. What plant breeders call pure-line cultivars are most aptly called "near" pure-line cultivars, because as researchers such as K. J. Frey observed, high mutation rates occur in such genotypes. Line cultivars have a very narrow genetic base and tend to be uniform in traits of interest (e.g., height, maturity). In cases of proprietary dispute, lines are easy to unequivocally identify.

Applications

Pure-line breeding is desirable for developing cultivars for certain uses:

1 Cultivars for mechanized production that must meet a certain specification for uniform operation by farm machines (e.g., uniform maturity, uniform height for location of economic part).

2 Cultivars developed for a discriminating market that puts a premium on visual appeal (e.g., uniform shape, size).
3 Cultivars for the processing market (e.g., demand for certain canning qualities, texture).
4 Advancing "sports" that appear in a population (e.g., a mutant flower for ornamental use).
5 Improving newly domesticated crops that have some variability.
6 The pure-line selection method is also an integral part of other breeding methods such as pedigree selection and bulk population selection.

Procedure

Overview

The pure-line selection in breeding entails repeated cycles of selfing, following the initial selection from a mixture of homozygous lines. Natural populations of self-pollinated species consist of mixtures of homozygous lines with transient heterozygosity originating from mutations and outcrossing.

Steps

Year 1 The first step is to obtain a variable base population (e.g., introductions, segregating populations from crosses, landrace) and space plant it in the first year, select, and harvest desirable individuals (Figure 16.3).

Year 2 Grow progeny rows of selected plants. Rogue out any variants. Harvest selected progenies individually. These are experimental strains.

Years 3–6 Conduct preliminary yield trials of the experimental strains including appropriate check cultivars.

Years 7–10 Conduct advanced yield trials at multiple locations. Release highest yielding line as new cultivar.

Genetic issues

Pure-line breeding produces cultivars with a narrow genetic base and hence less likely to produce stable

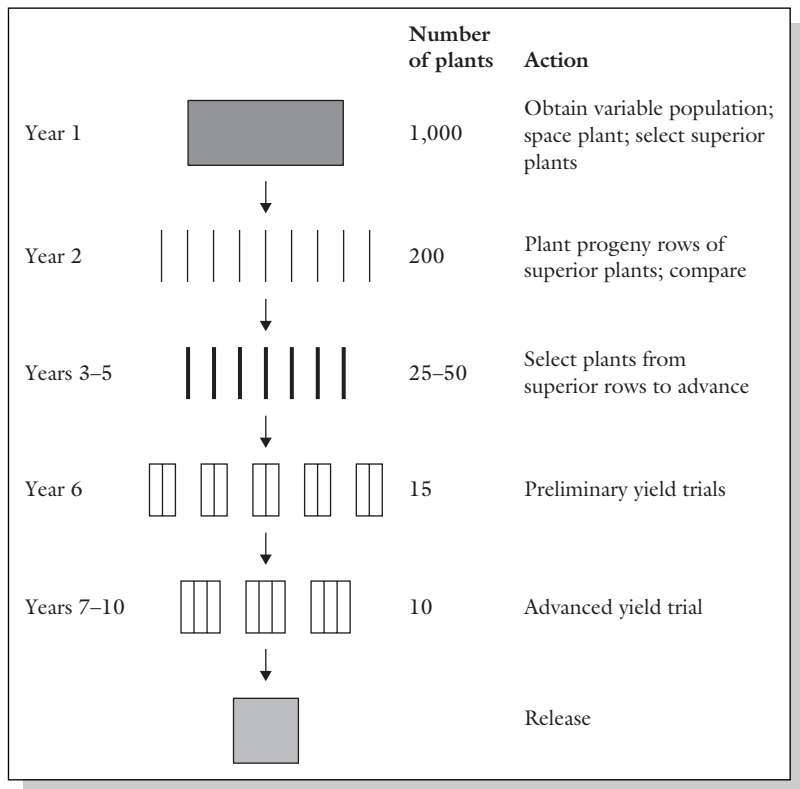

Figure 16.3 Generalized steps in breeding by pure-line selection.

yields over a wider range of environments. Such cultivars are more prone to being wiped out by pathogenic outbreaks. Because outcrossing occurs to some extent within most self-pollinated cultivars, coupled with the possibility of spontaneous mutation, variants may arise in commercial cultivars over time. It is tempting to select from established cultivars to develop new lines, an action that some view as unacceptable and unprofessional practice. As previously discussed, pure-line cultivars depend primarily on phenotypic plasticity for production response and stability across environments.

Advantages and disadvantages

Some of the major advantages and disadvantages of the application of the pure-line method for improving self-pollinated species are given here.

Advantages

1. It is a rapid breeding method.
2. The method is inexpensive to conduct. The base population can be a landrace. The population size selected is variable and can be small or large, depending on the objective.
3. The cultivar developed by this method has great "eye appeal" because of the high uniformity.
4. It is applicable to improving traits of low heritability, because selection is based on progeny performance.
5. Mass selection may include some inferior pure lines. In pure-line selection, only the best pure line is selected for maximum genetic advance.

Disadvantages

1. The purity of the cultivar may be altered through admixture, natural crossing with other cultivars, and mutations. Such off-type plants should be rouged out to maintain cultivar purity.
2. The cultivar has a narrow genetic base and hence is susceptible to devastation from adverse environmental factors, because of uniform response.
3. A new genotype is not created. Rather, improvement is limited to the isolation of the most desirable or best genotype from a mixed population.
4. The method promotes genetic erosion because most superior pure lines are identified and multiplied to the exclusion of other genetic variants.
5. Progeny rows take up more resources (time, space, funds).

Pedigree selection

Pedigree selection is a widely used method of breeding self-pollinated species (and even cross-pollinated species such as corn and other crops produced as hybrids). A key difference between pedigree selection and mass selection or pure-line selection is that hybridization is used to generate variability (for the base population), unlike the other methods in which production of genetic variation is not a feature. The method was first described by H. H. Lowe in 1927.

Key features

Pedigree selection is a breeding method in which the breeder keeps records of the ancestry of the cultivar. The base population, of necessity, is established by crossing selected parents, followed by handling an actively segregating population. Documentation of the pedigree enables breeders to trace parent–progeny back to an individual F_2 plant from any subsequent generation. To be successful, the breeder should be able to distinguish between desirable and undesirable plants on the basis of a single plant phenotype in a segregating population. It is a method of continuous individual selection after hybridization. Once selected, plants are reselected in each subsequent generation. This process is continued until a desirable level of homozygosity is attained. At that stage, plants appear phenotypically homogeneous.

The breeder should develop an effective, easy to maintain system of record keeping. The most basic form is based on numbering of plants as they are selected, and developing an extension to indicate subsequent selections. For example, if five crosses are made and 750 plants are selected in the F_2 (or list the first selection generation), a family could be designated 5-175 (meaning, it was derived from plant 175 selected from cross number 5). If selection is subsequently made from this family, it can be named, for example, 5-175-10. Some breeders include letters to indicate the parental sources or the kind of crop (e.g., NP-5-175-10), or some other useful information. The key is to keep it simple, manageable, and informative.

Applications

Pedigree selection is applicable to breeding species that allow individual plants to be observed, described, and harvested separately. It has been used to breed species including peanut, tobacco, tomato, and some cereals,

especially where readily identifiable qualitative traits are targeted for improvement.

General guides to selection following a cross

The success of breeding methods preceded by hybridization rest primarily on the parents used to initiate the breeding program. Each generation has genetic characteristics and is handled differently in a breeding program.

F_1 generation Unless in hybrid seed programs in which the F_1 is the commercial product, the purpose of the F_1 is to grow a sufficient F_2 population for selection. To achieve this, F_1 seed is usually space planted for maximum seed production. It is critical also to be able to authenticate hybridity and identity and remove seeds from self-pollination. Whenever possible, plant breeders use genetic markers in crossing programs.

F_2 generation Selection in the plant breeding program often starts in the F_2, the generation with the maximum genetic variation. The rate of segregation is higher if the parents differ by a larger number of genes. Generally, a large F_2 population is planted (2,000–5,000). Fifty percent of the genotypes in the F_2 are heterozygous and hence selection intensity should be moderate (about 10%) in order to select plants that would likely include those with the desired gene combinations. The actual number of plants selected depends on the trait (its heritability) and resources. Traits with high heritability are more effectively selected, requiring lower numbers than for traits with low heritability. The F_2 is also usually space planted to allow individual plants to be evaluated for selection. In pedigree selection, each selected F_2 plant is documented.

F_3 generation Seed from individual plants are progeny-rowed. This allows homozygous and heterozygous genotypes to be distinguished. The homozygosity in the F_3 is 50% less than in the F_2. The heterozygotes will segregate in the rows. The F_3 generation is the beginning of line formation. It is helpful to include check cultivars in the planting to help in selecting superior plants.

F_4 generation F_3 plants are grown in plant-to-row fashion as in the F_3 generation. The progenies become more homogeneous (homozygosity is 87.5%). Lines are formed in the F_4. Consequently, selection in the F_4 should focus more on progeny rather than on individuals plants.

F_5 generation Lines selected in the F_4 are grown in preliminary yield trials (PYTs). F_5 plants are 93.8% homozygous. These PYTs are replicated trials with at least two replications (depending on the amount of seed available). The seeding rate is the commercial rate (or as close as possible), receiving all the customary cultural inputs. Evaluation of quality traits and disease resistance can be included. The PYT should include check cultivars. The best performing lines are selected for advancing to the next stage in the breeding program.

F_6 generation The superior lines from F_5 are further evaluated in competitive yield trials or advanced yield trials (AYTs), including a check.

F_7 and subsequent generations Superior lines from F_6 are evaluated in AYTs for several years, at different locations, and in different seasons as desirable. Eventually, after F_8, the most outstanding entry is released as a commercial cultivar.

Procedure

Overview

The key steps in the pedigree selection procedure are:

1. Establish a base population by making a cross of selected parents.
2. Space plant progenies of selected plants.
3. Keep accurate records of selection from one generation to the next.

Steps

Year 1 Identify desirable homozygous parents and make about 20–200 crosses (Figure 16.4).

Year 2 Grow 50–100 F_1 plants including parents for comparison to authenticate its hybridity.

Year 3 Grow about 2,000–5,000 F_2 plants. Space plant to allow individual plants to be examined and documented. Include check cultivars for comparison. Desirable plants are selected and harvested separately keeping records of their identities. In some cases, it may be advantageous not to space plant F_2s to encourage competition among plants.

Year 4 Seed from superior plants are progeny-rowed in the F_3–F_5 generations, making sure to space plant the rows for easy record keeping. Selection at this stage is both within and

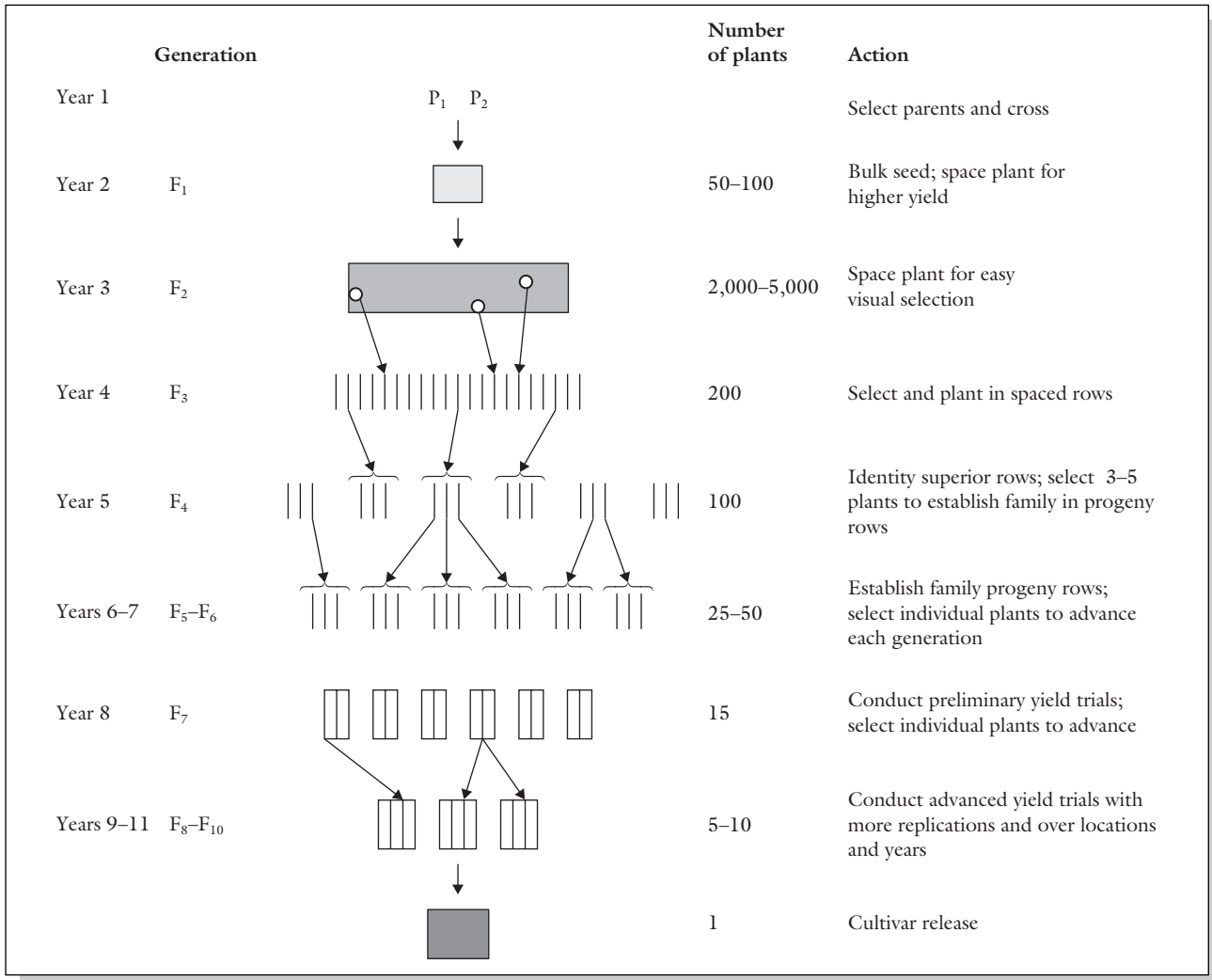

Figure 16.4 Generalized steps in breeding by pedigree selection.

between rows by first identifying superior rows and selecting 3–5 plants from each progeny to plant the next generation.

Year 5 By the end of the F_4 generation, there should be between 25–50 rows with records of the plant and row. Grow progeny of each selected F_3.

Year 6 Family rows are planted in the F_6 to produce experimental lines for preliminary yield trials in the F_7. The benchmark or check variety is a locally adapted cultivar. Several checks may be included in the trial.

Year 7 Advanced yield trials over locations, regions, and years are conducted in the F_8–F_{10} generations, advancing only superior experimental material to the next generation. Ultimately, the goal is to identify one or two lines that are superior to the check cultivars for release as a new cultivar. Consequently, evaluations at the advanced stages of the trial should include superior expression of traits that are deemed to be of agronomic importance for successful production of the particular crop (e.g., lodging resistance, shattering resistance, disease resistance). If a superior line is identified for release, it is put through the customary cultivar release process (i.e., seed increase and certification).

Comments

1. Growing parents, making a cross, and growing F_1 plants may take 1–2 years, depending on the facilities available for growing multiple experiments in a year (e.g., greenhouse) and the growing period of the crop.
2. The number of plants selected in the F_2 depends on resources available (labor, space, time), and can even be 10,000 plants.
3. F_3 family rows should contain a large enough number of plants (25–30) to permit the true family features to be evident so the most desirable plant(s) can be selected. Families that are distinctly inferior should be discarded, while more than one plant may be selected from exceptional families. However, generally, the number of plants advanced does not exceed the number of F_3 families.
4. From F_3 to F_5, selection is conducted between and within rows, identifying superior rows and selecting 3–5 of the best plants in each family. By F_5, only about 25–50 families are retained.
5. By F_5, plant density may reflect the commercial seeding rate. Further, the plants from this generation and future ones would be sufficiently homozygous to warrant conducting preliminary and, later, advanced yield trials.

Genetic issues

Detailed records are kept from one generation to the next regarding parentage and other characteristics of plants. The method allows the breeder to create genetic variability during the process. Consequently, the breeder can influence the genetic variation available by the choice of parents. The method is more conducive for breeding qualitative disease resistance, than for quantitative resistance. The product (cultivar) is genetically relatively narrow based but not as extremely so as in pure-line selection. The records help the breeder to advance only progeny lines with plants that exhibit genes for the desired traits.

Advantages and disadvantages

The pedigree method of breeding has advantages and disadvantages, the major ones include the following.

Advantages

1. Record keeping provides a catalog of genetic information of the cultivar unavailable from other methods.
2. Selection is based not only on phenotype but also on genotype (progeny row) making it an effective method for selecting superior lines from among segregating plants.
3. Using the records, the breeder is able to advance only the progeny lines in which plants that carry the genes for the target traits occur.
4. A high degree of genetic purity is produced in the cultivar, an advantage where such a property is desirable (e.g., certification of products for certain markets).

Disadvantages

1. Record keeping is slow, tedious, time-consuming, and expensive. It places pressure on resources (e.g., land for space planting for easy observation). Seeding and harvesting are tedious operations. However, modern research plot equipment for planting and harvesting are versatile and sophisticated to allow complex operations and record taking to be conducted, making pedigree selection easier to implement and hence be widely used. Large plant populations can now be handled without much difficulty.
2. The method is not suitable for species in which individual plants are difficult to isolate and characterize.
3. Pedigree selection is a long procedure, requiring about 10–12 years or more to complete, if only one growing season is possible.
4. The method is more suited for qualitative than for quantitative disease-resistance breeding. It is not effective for accumulating the number of minor genes needed to provide horizontal resistance.
5. Selecting in the F_2 (early generation testing) on the basis of quantitative traits such as yield may not be effective. It is more efficient to select among F_3 lines planted in rows than to select individual plants in the F_2.

Modifications

As previously indicated, the pedigree selection method is a continuous selection of individuals after hybridization. A discontinuous method (called the F_2 progenies test) has been proposed but is not considered practical enough for wide adoption. The breeder may modify the pedigree method to suit specific objectives and resources. Some specific ways are as follows:

1. The numbers of plants to select at each step may be modified according to the species, the breeding objective, and the genetics of the traits of interest, as well as the experience of the breeder with the crop, and resources available for the project.

2 The details of records kept are at the discretion of the breeder.
3 Off-season planting (e.g., winter nurseries), use of the greenhouse, and multiple plantings a year (where possible), are ways of speeding up the breeding process.

Early generation selection for yield in pedigree selection is not effective. This is a major objection to the procedure. Consequently, several modifications have been introduced by breeders to delay selection until later generations (e.g., F_5). Mass selection or bulk selection is practiced in the early generations.

Bulk population breeding

Bulk population breeding is a strategy of crop improvement in which the natural selection effect is solicited more directly in the early generations of the procedure by delaying stringent artificial selection until later generations. The Swede, H. Nilsson-Ehle, developed the procedure. H. V. Harlan and colleagues provided an additional theoretical foundation for this method through their work in barley breeding in the 1940s. As proposed by Harlan and colleagues, the bulk method entails yield testing of the F_2 bulk progenies from crosses and discarding whole crosses based on yield performance. In other words, the primary objective is to stratify crosses for selection of parents based on yield values. The current application of the bulk method has a different objective.

Key features

The rationale for delaying artificial selection is to allow natural selection pressure (e.g., abiotic factors such as drought, cold) to eliminate or reduce the productivity of less fit genotypes in the population. Just like the pedigree method, the bulk method also applies pure-line theory to segregating populations to develop pure-line cultivars. Genetic recombination in the heterozygous state cannot be used in self-pollinated species because self-pollination progressively increases homozygosity. By F_6 the homozygosity is about 98.9%. The strategy in plant breeding is to delay selection until there is a high level of homozygosity.

Applications

It is a procedure used primarily for breeding self-pollinated species, but can be adapted to produce inbred populations for cross-pollinated species. It is most suitable for breeding species that are normally closely spaced in production (e.g., small grains – wheat, barley). It is used for field bean and soybean. However, it is not suitable for improving fruit crops and many vegetables in which competitive ability is not desirable.

Procedure

Overview

After making a cross, several hundreds to several thousands of F_2 selections are planted at a predetermined (usually conventional rate), close spacing. The whole plot is bulk harvested. A sample of seed is used to plant another field block for the next selection, subjecting it to natural selection pressure through the next 2–3 generations. In the F_5, the plants are space planted to allow individual plant evaluation for effective selection. Preliminary yield trials may start in the F_7 followed by advanced yield trials, leading to cultivar release.

Steps

Year 1 Identify desirable parents (cultivars, single crosses, etc.) and make a sufficient number of crosses between them (Figure 16.5).

Year 2 Following a cross between appropriate parents, about 50–100 F_1 plants are planted and harvested as a bulk, after rouging out selfs.

Year 3 The seeds from the second year are used to plant a bulk plot of about 2,000–3,000 F_2 plants. The F_2 is bulk harvested.

Years 4–6 A sample of the F_2 seed is planted in bulk plots, repeating the steps for year 2 and year 3 until the F_4 is reached or when a desired level of homozygosity has been attained in the population. Space plant about 3,000–5,000 F_5 plants and select about 10% (300–500) superior plants for planting F_6 progeny rows.

Year 7 Select and harvest about 10% (30–50) progeny rows that exhibit genes for the desired traits for planting preliminary yield trails in the F_7.

Year 8 and later Conduct advanced yield trials from F_8 through F_{10} at multiple locations and regions, including adapted cultivars as checks. After identifying a superior line, it is put through the customary cultivar release process.

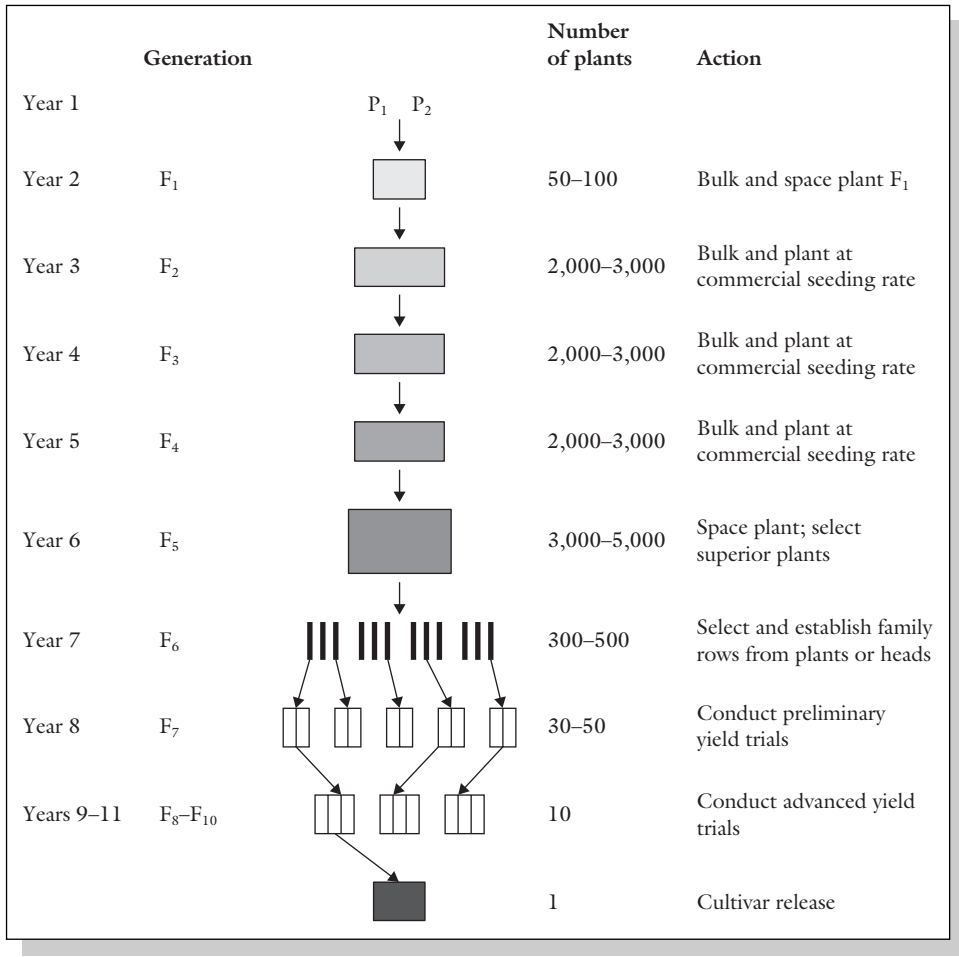

Figure 16.5 Generalized steps in breeding by bulk selection.

Comments

1. Space planting of the F_1 will increase the yield of F_2 seed.
2. The breeder may screen the bulk population under different natural environments in a rotation (e.g., soil condition – salinity, acidity; disease resistance; temperature – winter kill, etc.). There may be an increase in broad adaptation of the cultivar. However, care should be exercised to avoid the evaluation of plants under a condition that could eliminate genotypes that are of value at different sets of environmental conditions.
3. Screening for photoperiodic response is desirable and advantageous in the early stages to eliminate genotypes that are incapable of reproducing under the environmental conditions.
4. Natural selection may be aided by artificial selection. Aggressive and highly competitive but undesirable genotypes may be physically rogued out of the population to avoid increasing the frequency of undesirable genes, or to help select benign traits such as seed color or fiber length of cotton. Aiding natural selection also accelerates the breeding program.
5. The degree of selection pressure applied, its consistency, duration, and the heritability of traits, are all factors that impact the rate at which unadapted segregates are eliminated from the bulk population.

Genetic issues

Applying the theories of population genetics (see Chapter 7), repeated self-pollination, and fertilization will result in three key outcomes:

1 At advanced generations, the plants will be homozygous at nearly all loci.
2 The mean population performance will be improved as a result of natural selection.
3 Genotypes with good agricultural fitness will be retained in the population.

Bulk selection promotes intergenotypic competition. By allowing natural selection to operate on early generations, the gene frequencies in the population at each generation will depend upon:

1 The genetic potential of a genotype for productivity.
2 The competitive ability of the genotype.
3 The effect of the environment on the expression of a genotype.
4 The proportions and kinds of genotypes advanced to the next generation (i.e., sampling).

The effects of these factors may change from one generation to the next. More importantly, it is possible that desirable genotypes may be outcompeted by more aggressive undesirable genotypes. For example, tall plants may smother short desirable plants. It is not possible to predict which F_2 plant's progeny will be represented in the next generation, nor predict the genetic variability for each character in any generation.

The role of natural selection in bulk breeding is not incontrovertible. It is presumed to play a role in genetic shifts in favor of good competitive types, largely due to the high fecundity of competitive types. Such an impact is not hard to accept when traits that confer advantage through resistance to biotic and abiotic stresses are considered. For example, if the bulk populations were subjected to various environments (e.g., salinity, cold temperature, water logging, drought, photoperiod), fecundity may be drastically low for ill-adapted genotypes. These are factors that affect adaptation of plants. Some traits are more neutral in competition (e.g., disease resistance). If two genotypes are in competition, their survival depends on the number of seed produced by each genotype as well as the number of seeds produced by their progeny.

Using the natural relationship developed by W. Allard for illustration, the survival of an inferior genotype may be calculated as:

$$A_n = a \times S^{n-1}$$

where A_n = proportion of inferior genotypes, n = generation, a = initial proportion of the inferior genotype, and S = selection index. Given two genotypes, A (superior) and B (inferior), in equal proportions in a mixture (50% A : 50% B), and of survival capacities A = 1, B = 0.9, the proportion of the inferior genotype in F_5 would be:

$$A_5 = (0.5) \times (0.9)^{5-1}$$
$$= 0.3645 \text{ (or } 36.45\%)$$

This means the inferior genotype would decrease from 50% to 36.45% by F_5. Conversely, the proportion of the superior genotype would increase to 63.55%.

As previously indicated, the bulk selection method promotes intergenotypic competition; it is important to point out that the outcome is not always desirable because a more aggressive inferior genotype may outcompete a superior (desirable) but poor competitor. In a classic study by C. A. Suneson, equal mixtures (25%) of four barley cultivars were followed. After more than five generations, the cultivar "Atlas" was represented by 88.1%, "Club Mariot" by 11%, "Hero" by 1%, while "Vaughn" was completely eliminated. However, in pure stands, "Vaughn" outyielded "Atlas". It may also be said that if the genotypes whose frequency in the population increased over generations are the ones of agronomic value (i.e., desired by the breeder), then the competition in bulking is advantageous to plant breeding. The effect of natural selection in the bulk population can be positive or negative, and varies according to the traits of interest, the environment under which the population is growing, and the degree of intergenotypic competition (spacing among plants). If there is no competition between plants, genotype frequencies would not be changed significantly. Also, the role of natural selection in genetic shifts would be less important when the duration of the period is smaller (6–10 generations), as is the case in bulk breeding. This is so because natural selection acts on the heterozygotes in the early generations. However, the goal of bulk breeding is to develop pure lines. By the time the cultivar is released, the breeding program would have ended, giving natural selection no time to act on the pure lines.

Advantages and disadvantages

Some of the key advantages and disadvantages of bulk breeding method are as follows.

Advantages

1 It is simple and convenient to conduct.
2 It is less labor intensive and less expensive in early generations.

3 Natural selection may increase frequency of desirable genotypes by the end of the bulking period.
4 It is compatible with mass selection in self-pollinated species.
5 Bulk breeding allows large amounts of segregating materials to be handled. Consequently, the breeder can make and evaluate more crosses.
6 The cultivar developed would be adapted to the environment, having been derived from material that had gone through years of natural selection.
7 Single-plant selections are made when plants are more homozygous, making it more effective to evaluate and compare plant performance.

Disadvantages

1 Superior genotypes may be lost to natural selection, while undesirable ones are promoted during the early generations.
2 It is not suited to species that are widely spaced in normal production.
3 Genetic characteristics of the populations are difficult to ascertain from one generation to the next.
4 Genotypes are not equally represented in each generation because all the plants in one generation are not advanced to the next generation. Improper sampling may lead to genetic drift.
5 Selecting in off-season nurseries and the greenhouse may favor genotypes that are undesirable in the production region where the breeding is conducted, and hence is not a recommended practice.
6 The procedure is lengthy, but cannot take advantage of off-season planting.

Modifications

Modifications of the classic bulk breeding method include the following:

1 The breeder may impose artificial selection sooner (F_3 or F_4) to shift the population toward an agriculturally more desirable type.
2 Rouging may be conducted to remove undesirable genotypes prior to bulking.
3 The breeder may select the appropriate environment to favor desired genotypes in the population. For example, selecting under disease pressure would eliminate susceptible individuals from the population.
4 Preliminary yield trials may be started even while the lines are segregating in the F_3 or F_4.
5 The **single-seed descent** method may be used at each generation to reduce the chance of genetic drift. Each generation, a single seed is harvested from each plant to grow the next bulk population. The dense planting makes this approach problematic in locating individual plants.
6 **Composite cross bulk population** breeding, also called the **evolutionary method of breeding**, was developed by C. A. Suneson and entails systematically crossing a large number of cultivars. First, pairs of parents are crossed, then pairs of F_1s are crossed. This continues until a single hybrid stock containing all parents is produced. The method has potential for crop improvement, but it takes a very long time to complete.

Single-seed descent

The method of single-seed descent was born out of a need to speed up the breeding program by rapidly inbreeding a population prior to beginning individual plant selection and evaluation, while reducing a loss of genotypes during the segregating generations. The concept was first proposed by C. H. Goulden in 1941 when he attained the F_6 generation in 2 years by reducing the number of generations grown from a plant to one or two, while conducting multiple plantings per year, using the greenhouse and off-season planting. H. W. Johnson and R. L. Bernard described the procedure of harvesting a single seed per plant for soybean in 1962. However, it was C. A. Brim who in 1966 provided a formal description of the procedure of single-seed descent, calling it a **modified pedigree method**.

Key features

The method allows the breeder to advance the maximum number of F_2 plants through the F_5 generation. This is achieved by advancing one randomly selected seed per plant through the early segregating stages. The focus on the early stages of the procedure is on attaining homozygosity as rapidly as possible, without selection. Discriminating among plants starts after attainment of homozygosity.

Applications

Growing plants in the greenhouse under artificial conditions tends to reduce flower size and increase cleistogamy. Consequently, single-seed descent is best for self-pollinated species. It is effective for breeding small

grains as well as legumes, especially those that can tolerate close planting and still produce at least one seed per plant. Species that can be forced to mature rapidly are suitable for breeding by this method. It is widely used in soybean breeding to advance the early generation. One other major application of single-seed descent is in conjunction with other methods.

Procedure

Overview

A large F_1 population is generated to ensure adequate recombination among parental chromosomes. A single seed per plant is advanced in each subsequent generation until the desired level of inbreeding is attained. Selection is usually not practiced until F_5 or F_6. Then, each plant is used to establish a family to help breeders in selection and to increase seed for subsequent yield trials.

Steps

Year 1	Crossing is used to create the base population. Cross selected parents to generate an adequate number of F_1 for the production of a large F_2 population.
Year 2	About 50–100 F_1 plants are grown in a greenhouse in the ground, on a bench, or in pots. They may also be grown in the field. Harvest identical F_1 crosses and bulk.
Year 3	About 2,000–3,000 F_2 plants are grown. At maturity, a single seed per plant is harvested and bulked for planting F_3. Subsequently, the F_2 plants are spaced enough to allow each plant to produce only a few seeds.
Years 4–6	Single pods per plant are harvested to plant the F_4. The F_5 is space planted in the field, harvesting seed from only superior plants to grow progeny rows in the F_6 generation.
Year 7	Superior rows are harvested to grow preliminary yield trials in the F_7.
Year 8 and later	Yield trials are conducted in the F_8–F_{10} generations. The most superior line is increased in the F_{11} and F_{12} as a new cultivar.

Comments

1. If the sample is too small, superior genetic combinations may be lost because only one seed from each plant is used.
2. It may be advantageous to use progeny rows prior to yield testing to produce sufficient seed as well as to help in selecting superior families.
3. The breeder may choose to impose some artificial selection pressure by excluding undesirable plants from contributing to the subsequent generations (in the early generations). This is effective for qualitative traits.
4. Record keeping is minimal and so are other activities such as harvesting, especially in the early generations.

Genetic issues

Each individual in the final population is a descendent from a different F_2 plant. Each of these plants undergoes a decrease in heterozygosity at a rapid rate, each generation. Barring the inability of a seed to germinate or a plant to set seed, the effect of natural selection is practically non-existent in the single-seed descent procedure. Only one seed per plant is advanced, regardless of the number produced. That is, a plant producing one seed is as equally represented in the next generation as one producing 1,000 seeds. Selection is conducted on homozygous plants rather than segregating material. An efficient early generation testing is needed to avoid genetic drift of desirable alleles. Single-seed descent is similar to bulk selection in that the F_6/F_7 comprises a large number of homozygous lines, prior to selection among progenies. A wide genetic diversity is carried on to relatively advanced generations (F_6/F_7).

Advantages and disadvantages

Single-seed descent has certain advantages and disadvantages, the major ones including the following.

Advantages

1. It is an easy and rapid way to attain homozygosity (2–3 generations per year).
2. Small spaces are required in early generations (e.g., can be conducted in a greenhouse) to grow the selections.
3. Natural selection has no effect (hence it can not impose an adverse impact).
4. The duration of the breeding program can be reduced by several years by using single-seed descent.

5 Every plant originates from a different F_2 plant, resulting in greater genetic diversity in each generation.
6 It is suited to environments that do not represent those in which the ultimate cultivar will be commercially produced (no natural selection imposed).

Disadvantages

1 Natural selection has no effect (hence no benefit from its possible positive impact).
2 Plants are selected based on individual phenotype not progeny performance.
3 An inability of seed to germinate or a plant to set seed may prohibit every F_2 plant from being represented in the subsequent population.
4 The number of plants in the F_2 is equal to the number of plants in the F_4. Selecting a single seed per plant runs the risks of losing desirable genes. The assumption is that the single seed represents the genetic base of each F_2. This may not be true.

Modifications

The procedure described so far is the classic single-seed descent breeding method. There are two main modifications of this basic procedure. The multiple seed procedure (or **modified single-seed descent**) entails selecting 2–4 seeds per plant, bulking and splitting the bulk into two, one for planting the next generation, and the other half held as a reserve. Because some soybean breeders simply harvest one multiseeded pod per plant, the procedure is also referred to by some as the **bulk pod method**.

Another modification is the **single hill method** in which progeny from individual plants are maintained as separate lines during the early generations by planting a few seeds in a hill. Seeds are harvested from the hill and planted in another hill the next generation. A plant is harvested from each line when homozygosity is attained.

Industry highlights

Barley breeding in the United Kingdom

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Table 1 Characters listed in the current UK recommended lists of barley (www.hgca.com).

Character	Spring barley	Winter barley
Yield (overall and regional with fungicide)	Yes	Yes
Yield without fungicide	Yes	Yes
Height	Yes	Yes
Lodging resistance	Yes	Yes
Brackling resistance	Yes	
Maturity	Yes	Yes
Winter hardiness		Yes
Powdery mildew resistance	Yes	Yes
Rhynchosporium resistance	Yes	Yes
Yellow rust resistance	Yes	Yes
Brown rust resistance	Yes	Yes
Net blotch resistance		Yes
BaYMV complex resistance		Yes
BYDV resistance	Yes	
Grain nitrogen content	Yes	Yes
Hot water extract	Yes	Yes
Screenings (2.25 and 2.5 mm)	Yes	Yes
Specific weight	Yes	Yes

Targets

Barley breeding in the UK aims to produce new cultivars that offer an improvement in one or more of the key characters for the region (Table 1). New cultivars must have a good yield, preferably in excess of the current established cultivars, if targeted solely at the feed market. To be accepted for malting use, a new cultivar must offer improvement in one or more key facets of malting quality, primarily hot water extract, with no major defects in, for instance, processability characters. Additionally, new cultivars must have minimum levels of disease resistance, which equates to being no worse than moderately susceptible, to the key diseases listed in Table 1.

Crossing to commercialization

Barley breeders therefore design crosses in which the parents complement each other for these target characters and attempt to select out recombinants that offer a better balanced overall phenotype. Whilst a wide cross may offer a better chance of producing superior

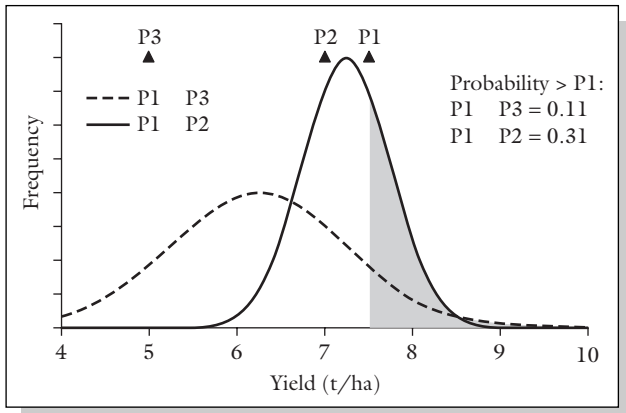

Figure 1 Frequency distribution of two crosses with a common parent (P1) and alternative second parents (P2 and P3). P2 is a slightly lower yielding parent, thus progeny from the cross will have a high mid-parent value and small variation. P3 is comparatively high-yielding unadapted parent and the cross has a lower mid-parent value but much greater variance. Areas under the shaded portion of both curves represent the fraction selected for high-yield potential (> P1). Thus, while the extreme recombinant of P1 × P3 has a greater yield potential than that of P1 × P2, the probability of identifying superior lines for just this one character is far greater for the latter.

recombinants, most barley breeders in the UK concentrate on narrow crosses between elite cultivars. The main reason for doing so is that a narrow cross between elite lines is more likely to produce a high midparental value for any one character and so the proportion of desirable recombinants is thus far greater in the narrow cross than in the wide cross (Figure 1). Thus, the chances of finding a desirable recombinant for a complex character such as yield in the wide cross is low and the chances of combining it with optimum expression for all the other characters is remote. As breeders are still making progress using such a narrow crossing strategy, it is possible that there is still an adequate level of genetic diversity within the elite barley gene pool in the UK. A similar phenomenon has been observed in barley breeding in the USA where progress has been maintained despite a narrow crossing strategy (Rasmusson & Phillips 1997). Rae et al. (2005) genotyped three spring barley cultivars ("Cocktail", "Doyen", and "Troon") on the 2005 UK recommended list with 35 simple sequence repeat (SSR) markers and found sufficient allelic diversity to produce over 21 million different genotypes. It would therefore appear that the breeding challenge is not so much to generate variation as to identify the best recombinants.

The progress of a potential new barley cultivar in the UK, in common with that of other cereals, proceeds through a series of filtration tests (Figure 2) and the time taken to pass through all but the first is strictly defined. The opportunity to reduce the time taken for breeders' selections is fairly limited given that the multiplication of material for, and the conducting of single- and multisite trials, takes at least 3 years, irrespective of whether one uses out-of-season nurseries for shuttle breeding for the spring crop or doubled haploidy (DH) or single-seed descent (SSD) for the winter crop. The length of the breeding cycle is thus fairly well defined with occasional reduction by a year when a cultivar from a highly promising cross is speculatively advanced by a breeder. A breeder may also delay submitting a line for official trials for an extra season's data but breeders now aim to submit the majority of their lines to official trials within 4–5 years of making a cross. Given that many breeders would have begun recrossing such selections by this stage of their development, the approximate time for the breeding cycle in the UK is 4 years.

During the 2 years of national list trials (NLTs), potential cultivars are tested for distinctness, uniformity, and stability (DUS) using established botanical descriptors. A submission therefore has to be distinct from any other line on the National List and not have more than a permitted level of off-types, currently equivalent to a maximum of three in 100 ear rows. Lines are tested over more than 1 year to ensure that they are genetically stable and do not segregate in a subsequent generation. DUS tests are carried out by detailed examination of 100 ear rows and three bulk plots (approximately 400 plants in total) submitted by the breeder. Thirty-three characters are examined routinely and there are three special and 59 approved additional characters. At the same time as plot trials are carried out to establish whether the submission has value for cultivation and use (VCU), the VCU and DUS submissions are checked to verify that they are the same. Occasionally, a submission may fail the DUS test in NLT_1 in which case the breeder has the option of submitting a new stock for a further 2 years of testing. Generally, the VCU results are allowed to stand and a cultivar can be entered into the recommended list trials (RLTs) before it has passed the DUS test in the anticipation that it will have succeeded by the time a recommendation decision has to be made. Full details can be obtained from www.defra.gov.uk/planth/pvs/VCU_DUS.htm.

The UK barley breeding community

The Plant Varieties and Seeds Act of 1964, which enabled plant breeders to earn royalties on certified seed produced for their cultivars, led to a dramatic increase in breeding activity in the UK. Formerly, it was largely the province of state-funded improvement programs such as that of the Plant Breeding Institute (PBI), Cambridge, which had produced the highly successful spring cultivar "Proctor". The increase in breeding activity in the 1970s and early 1980s was largely as a result of a dramatic expansion in the commercial sector, initially led by Miln Marsters of Chester, UK, who produced "Golden Promise", which dominated Scottish spring barley production for almost two decades. The two sectors coexisted until the privatization of the breeding activity at PBI

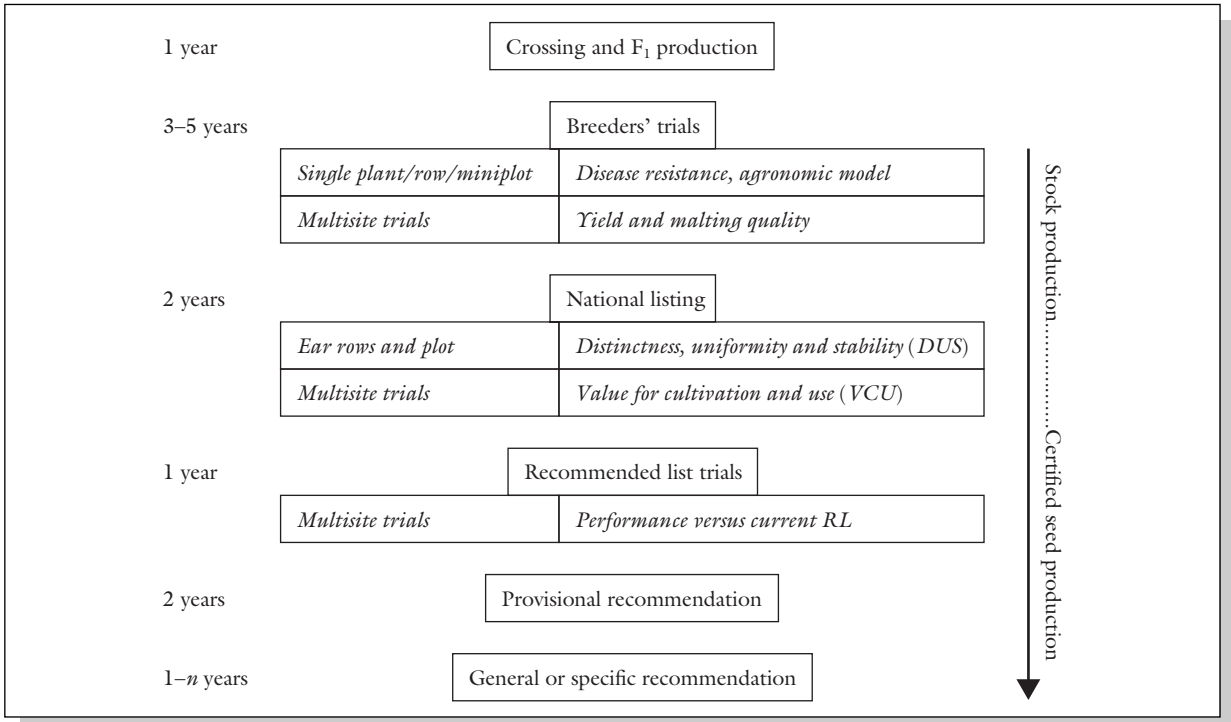

Figure 2 Breakdown of the phases in the development of a successful new cultivar from crossing to commercialization, with the timescale for each step. The exact nature of the scheme adopted in breeders' trials varies according to the breeder and crop type, but is either based upon a version of the pedigree or a doupled haploid system. A cultivar may persist on the recommended list (RL) for *n* years, where *n* is the number of years where there is a significant demand for it.

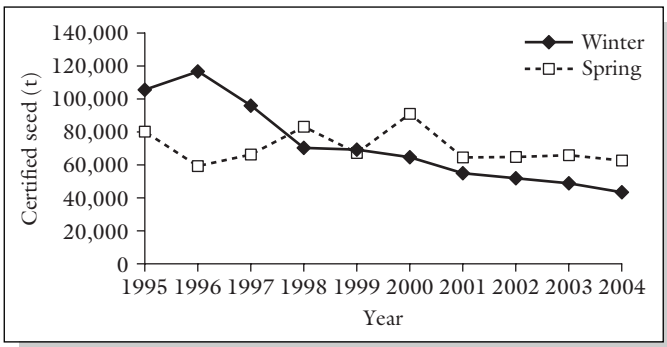

Figure 3 Tonnes of certified barley seed produced in the UK from 1995 to 2004.

and the state marketing arm, the National Seed Development Organization, together with a change in government policy led to the withdrawal of the public sector from barley breeding in the UK. Barley breeding in the commercial sector in the UK is highly competitive with currently five UK-based crossing and selection programs. A number of other companies have their own selection programs based in the UK and many continental breeders have agency agreements for the testing and potential marketing of their products. For example, 41 spring and 34 winter barley lines were submitted for NLT_1 testing for harvest in 2004 and these were derived from 16 different breeders.

The amount of certified seed produced for each cereal variety in the UK is published by the National Institute of Agricultural Botany. The total annual production of certified barley seed has been in decline since its peak of over 250,000 tonnes in 1987, and has declined by 43% since 1995 with most due to a reduction in winter barley seed (Figure 3). There are a number of potential reasons for this, such as an increase in farm-saved seed, but the principal feature has been a marked decrease in winter barley cropping over the period whereas spring barley has remained fairly static and winter wheat has increased. Over this period, certified seed production has exceeded 100,000 tonnes for two spring ("Opti" and "Chariot") and two winter ("Regina" and "Pearl") barley cultivars and these can be considered notable market successes. There has been substantial production of a number of others but

total production exceeded 25,000 tonnes for only six spring and seven winter barley cultivars. When one considers that over 830 lines were submitted for NLTs over this period, the overall success rate is 1.6%. Nevertheless, real breeding progress is being made. Using yield data from the recommended list trials from 1993 to 2004 to estimate the mean yield of each recommended cultivar and then regressing that data against the year that it was first recommended, revealed that genetic progress was in the order of 1% per annum (Rae et al. 2005).

Impact of molecular markers

The first whole genome molecular maps of barley were published in 1991 (Graner et al. 1991; Heun et al. 1991) and were closely followed by QTL maps in 1992 (Heun 1992) and 1993 (Hayes et al. 1993) with well over 40 barley mapping studies now in the public domain. Despite this apparent wealth of information, barley breeders in the UK are largely relying on conventional phenotypic selection to maintain this progress. This is in marked contrast to the highly successful use of marker-assisted selection (MAS) in the Australian barley program (Langridge & Barr 2003), which is probably a reflection of the different breeding strategies in the two countries. In the UK, improvement is being achieved in the elite gene pool, as noted above, whereas MAS has been deployed in an introgression breeding strategy in Australia. Given that most barley mapping studies have concentrated on diverse crosses to maximize polymorphism and facilitate map construction, there are very few published QTL studies that are relevant to current UK barley breeding strategies. Surveying results from eight different barley mapping populations (Thomas 2003), found that there were very few instances where QTLs were co-located for three or more crosses for important characters such as yield and hot water extract.

Major gene targets

Markers have been developed for a number of known major genes and could potentially be deployed in MAS by UK breeders. Many of these major gene targets are, however, disease resistances, many of which have been defeated by matching virulence in the corresponding pathogen population. UK barley breeders have been required to select for at least some resistance to the key foliar pathogens listed in Table 1 since the introduction of minimum standards, and have accordingly developed efficient phenotypic screens. There are exceptions, most notably the barley yellow mosaic virus (BaYMV) complex, which is transmitted by infection of the roots with the soil-borne fungus vector *Polymixa graminis*. A phenotypic screen therefore requires an infected site and the appropriate environment for infection and expression. Phenotypic screening can be expensive if a breeder is distant from an infected site and is subject to potential misclassification.

Resistance due to the *rym4* allele was initially found in "Ragusa" and was effective against BaYMV strain 1 and a number of cultivars carrying this allele have been developed, initially by phenotypic screening. Markers to select for this resistance have also been developed, beginning with the RFLP (restricted fragment length polymorphism) probe MWG838 (Graner & Bauer 1993), later converted to an STS (sequence-tagged site) (Bauer & Graner 1995), and were used in some breeding programs in the UK and Europe. BaYMV strain 2, which became more frequent in the 1990s, could overcome the *rym4* resistance, but another resistance, *rym5*, was identified in "Mokusekko 3" as being effective against both strains. This resistance was co-located with *rym4* and the SSR marker Bmac29 was found to be linked to it (Graner et al. 1999). Bmac29 could not only distinguish between resistant and susceptible alleles but also between the *rym4* and *rym5* alleles derived from "Ragusa" and "Mokusekko 3", respectively. However, as it is 1.3 cM from the gene locus, it is not effective in a wide germplasm pool as *Hordeum spontaneum* lines predicted to be resistant by the marker were found to be susceptible (R. P. Ellis, unpublished data). Bmac29 has, however, proved to be particularly effective for UK, and European, barley breeders as they are working with a narrow genetic base and just the two sources of resistance. Other resistance loci have been identified together with suitable markers to deploy in a pyramiding strategy in an attempt to provide durable resistance (Ordon et al. 2003). They provide a clear example of how the use of markers in MAS has evolved together with the pathogen.

Another example relates to a particular requirement of the Scotch whisky distilling industry. In grain and certain malt whisky distilleries, a breakdown product of the gynogenic glycoside epiheterodendrin can react with copper in the still to form the carcinogen ethyl carbamate, which can be carried over into the final spirit in distilling. This has lead to a demand for barley cultivars that do not produce epiheterodendrin. The character is controlled by a single gene with the non-producing allele originating in the mildew resistance donor "Arabische" used in the derivation of the cultivar "Emir". The phenotypic assay for the character involves the use of hazardous chemicals, and the finding of a linked SSR marker (Bmac213) offered a simpler and safer alternative (Swanston et al. 1999). The distance between the gene locus and the marker (6 cM) meant that, in contrast to Bmac29, Bmac213 was not reliable in the cultivated gene pool. For instance, the cultivar "Cooper" and its derivatives possess the non-producing allele yet are producers. However, the marker could still be used when the parents of a cross were polymorphic for both the phenotype and the marker. Recently, a candidate gene has been identified and markers used for reliable identification of non-producers have been developed (P. Hedley, personal communication).

QTL targets

Currently, UK barley breeders do not use MAS for any other malting quality targets. A QTL for fermentability was detected in a cross between elite UK genotypes (Swanston et al. 1999) but the increasing allele was derived from the parent with relatively poor

malting quality. When this QTL was transferred into a good malting quality cultivar, the results were inconclusive (Meyer et al. 2004), probably because the effect of the gene was more marked in a poor quality background and any extra activity due to it was superfluous in a good quality background. This highlights one of the problems in developing MAS for complex characters such as yield and malting quality. Results from an inappropriate gene pool may well not translate to a target gene pool and it is therefore essential that QTL studies are carried out in the appropriate genetic background.

Future prospects

The genotyping of entries from Danish registration trials coupled with associations of markers with yield and yield stability phenotypes demonstrated that QTLs can be detected in the elite gene pool (Kraakman et al. 2004) but the findings need validation before the markers can be used in MAS. At the Scottish Crop Research Institute, we will be undertaking extensive genotyping of UK RLT entries over the past 12 years in collaboration with the University of Birmingham, the National Institute of Agricultural Botany, the Home Grown Cereals Authority, UK, barley breeders, and representatives of the malting, brewing, and distilling industries in a project funded by the Defra Sustainable Arable LINK scheme. The RLT phenotypic data set represents an extensive resource that can discriminate between the fine differences in elite cultivars and will facilitate the identification of meaningful associations within the project for validation and potential use in MAS. How MAS is then utilized by commercial breeders in the UK might well vary but could range from early generation selection from an enriched germplasm pool upon which phenotypic selection can be concentrated, to identification of candidate submission lines carrying target traits.

Acknowledgments

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Backcross breeding

The application of this method in plants was first proposed by H. V. Harlan and M. N. Pope in 1922. In principle, **backcross breeding** does not improve the genotype of the product, except for the substituted gene(s).

Key features

The rationale of backcross breeding is to replace a specific undesirable gene with a desirable alternative, while preserving all other qualities (adaptation, productivity, etc.) of an adapted cultivar (or breeding line). Instead of inbreeding the F_1 as is normally done, it is repeatedly crossed with the desirable parent to retrieve (by "modified inbreeding") the desirable genotype. The adapted and highly desirable parent is called the **recurrent parent** in the crossing program, while the source of the desirable gene missing in the adapted parent is called the **donor parent**. Even though the chief role of the donor parent is to supply the missing gene, it should not be significantly deficient in other desirable traits. An inferior recurrent parent will still be inferior after the gene transfer.

Applications

The backcross method of breeding is best suited to improving established cultivars that are later found to be deficient in one or two specific traits. It is most effective and easy to conduct when the missing trait is qualitatively (simply) inherited, dominant, and produces a phenotype that is readily observed in a hybrid plant. Quantitative traits are more difficult to breed by this method. The procedure for transferring a recessive trait is similar to that for dominant traits, but entails an additional step.

Backcrossing is used to transfer entire sets of chromosomes in the foreign cytoplasm to create a cytoplasmic male-sterile (CMS) genotype that is used to facilitate hybrid production in species including corn, onion, and wheat. This is accomplished by crossing the donor (of the chromosomes) as male until all donor chromosomes are recovered in the cytoplasm of the recurrent parent.

Backcrossing is also used for the introgression of genes via wide crosses. However, such programs are often lengthy because wild plant species possess significant amounts of undesirable traits. Backcross breeding can also be used to develop **isogenic lines** (genotypes that differ only in alleles at a specific locus) for traits (e.g., disease resistance, plant height) in which phenotypes contrast. The method is effective for breeding when the expression of a trait depends mainly on one pair of genes, the heterozygote is readily identified, and the species is self-fertilizing. Backcrossing is applicable in the development of multilines (discussed next).

Procedure

Overview

To initiate a backcross breeding program, the breeder crosses the recurrent parent with the donor parent. The F_1 is grown and crossed with the recurrent parent again. The second step is repeated for as long as it takes to recover the characteristics of the recurrent parent. This may vary from two to five cycles (or more in some cases) depending on how easy the expression of the transferred gene is to observe, how much of the recurrent parental genotype the breeder wants to recover, and the overall acceptability of the donor parent. A selection pressure is imposed after each backcross to identify and discard the homozygous recessive individuals. Where the desired trait is recessive, it will be necessary to conduct a progeny test to determine the genotype of a backcross progeny before continuing with the next cross.

Steps: dominant gene transfer

Year 1 Select the donor (*RR*) and recurrent parent (*rr*) and make 10–20 crosses. Harvest the F_1 seed (Figure 16.6).

Year 2 Grow F_1 plants and cross (backcross) with the recurrent parent to obtain the first backcross (BC_1).

Years 3–7 Grow the appropriate backcross (BC_1–BC_5) and backcross to the recurrent parent as female. Each time, select about 30–50 heterozygous parents (backcrosses) that most resemble the recurrent parent to be used in the next backcross. The recessive genotypes are discarded after each backcross. The breeder should use any appropriate screening techniques to identify the heterozygotes (and discard the homozygous recessives). For disease-resistance breeding, artificial epiphytotic conditions are created. After six backcrosses, the BC_5 should very closely resemble the recurrent parent and express the donor trait. As generations advance, most plants would be increasingly more like the adapted cultivar.

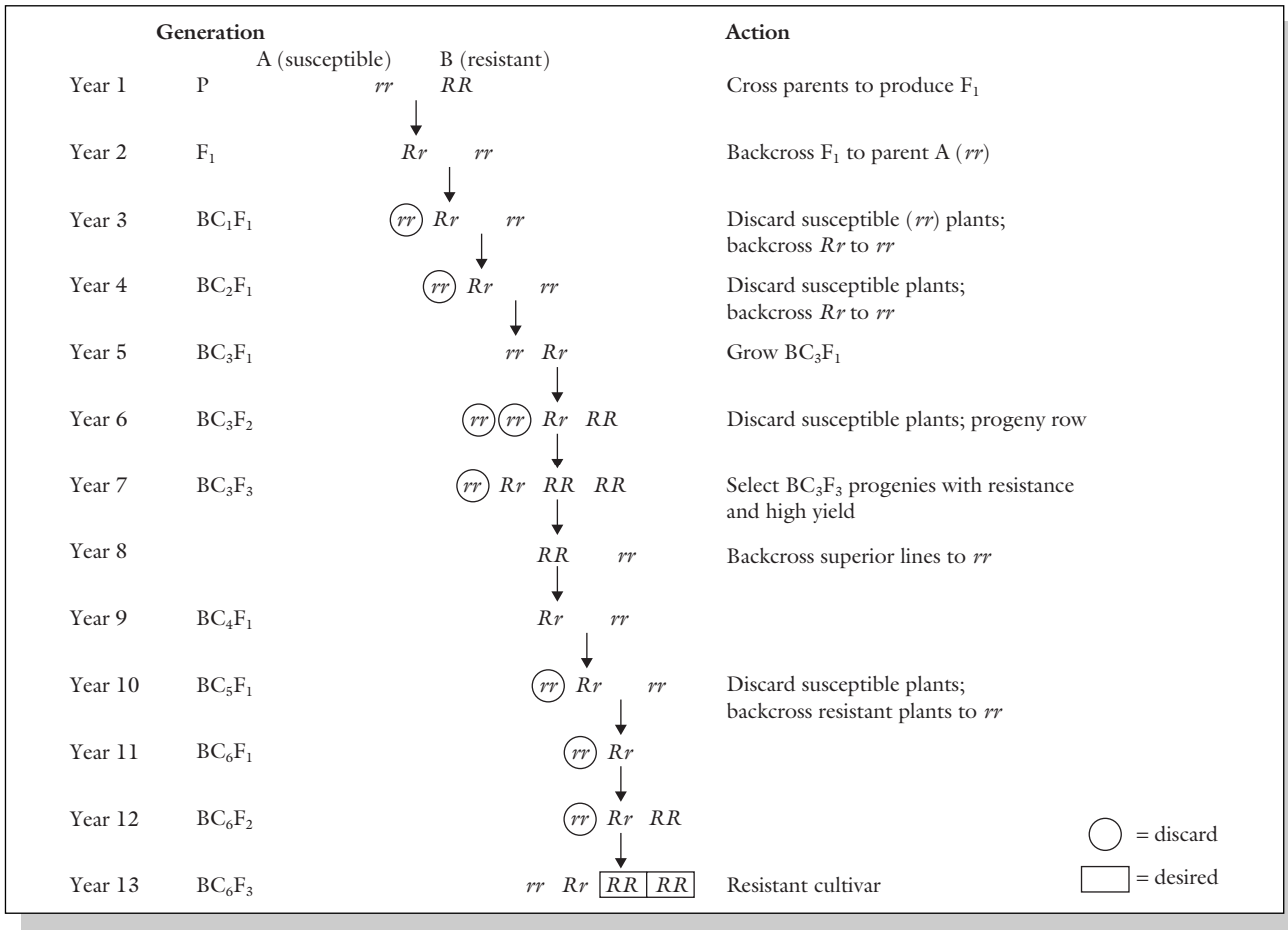

Figure 16.6 Generalized steps in breeding a dominant trait by the backcross method. The exact steps vary among breeding programs.

Year 8 Grow BC_5F_1 plants to be selfed. Select several hundreds (300–400) desirable plants and harvest them individually.

Year 9 Grow BC_5F_2 progeny rows. Identify and select about 100 desirable non-segregating progenies and bulk.

Year 10 Conduct yield tests of the backcross with the recurrent cultivar to determine equivalence before releasing.

Comments: dominant gene transfer

The steps for transferring a dominant gene are straightforward. Following the first cross between the parents, phenotypic selection is adequate for selecting plants that exhibit the target trait. Recessive genotypes are discarded. The recurrent parent traits are not selected at this stage. The next cross is between the selected F_1 and the recurrent parent. This step is repeated for several cycles (BC_n). After satisfactory recovery of the recurrent parent, the selected plant (BC_nF_1) will be homozygous for other alleles but heterozygous for the desired traits. The last backcross is followed by selfing to stabilize the desired gene in the homozygous state. All homozygous (BC_nF_2) recessive segregates are discarded.

Steps: recessive gene transfer

Years 1–2 These are the same as for dominant gene transfer. The donor parent has the recessive desirable gene (Figure 16.7).

Year 3 Grow BC_1F_1 plants and self, harvest, and bulk the BC_1F_2 seed. In disease-resistance breeding, all BC_1s will be susceptible.

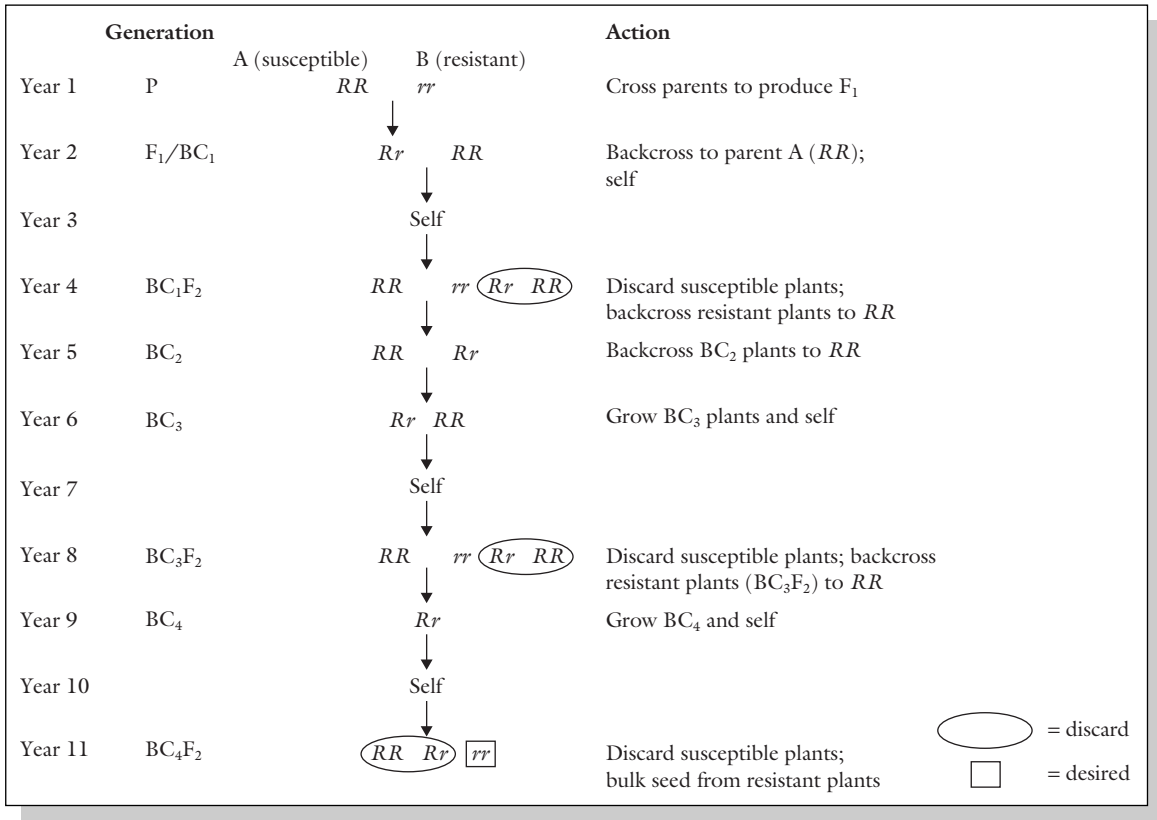

Figure 16.7 Generalized steps in breeding a recessive trait by the backcross method. The exact steps vary among breeding programs.

Year 4 Grow BC_1F_2 plants and screen for desirable plants. Backcross 10–20 plants to the recurrent parent to obtain BC_2F_2 seed.

Year 5 Grow BC_2 plants. Select 10–20 plants that resemble the recurrent parent and cross with the recurrent parent.

Year 6 Grow BC_3 plants, harvest and bulk the BC_3F_2 seed.

Year 7 Grow BC_3F_2 plants, screen, and select the desirable plants. Backcross 10–20 plants with the recurrent parent.

Year 8 Grow BC_4 plants, harvest, and bulk the BC_4F_2 seed.

Year 9 Grow BC_4F_2 plants, screen, and select the desirable plants. Backcross 10–20 plants with the recurrent parent.

Year 10 Grow BC_5 plants, harvest, and bulk the BC_5F_2 seed.

Year 11 Grow BC_5F_2 plants, screen, and backcross.

Year 12 Grow BC_6 plants, harvest, and bulk the BC_6F_2 seed.

Year 13 Grow BC_6F_2 plants and screen; select 400–500 plants and harvest separately for growing progeny rows.

Year 14 Grow progenies of selected plants, screen, and select about 100–200 uniform progenies; harvest and bulk the seed.

Years 15–16 Follow the procedure as in breeding for a dominant gene.

The key difference between the transfer of dominant and recessive alleles is that in the latter case, phenotypic identification is not possible after a cross. Each cross needs to be followed by selfing so that the progeny with the homozygous recessive genotype can be identified and backcrossed to the recurrent parent.

Comments: recessive gene transfer

1 Backcrossing does not have to be conducted in the environment in which the recurrent parent is adapted because all that is needed is to be able to identify and select the target trait.

2 Extensive advanced testing is not necessary in a backcross because the new cultivar already resembles the adapted cultivar, except for the newly incorporated trait.
3 It is possible to transfer two or more genes by simultaneous selection among the progeny. This undertaking requires a larger population than would be necessary if two genes are transferred independently.
4 Introgression of genes from weedy, adapted, exotic, or wild germplasm is possible by backcrossing. However, such transfers often take longer than typical transfers, because of the time needed to remove the undesirable agronomic traits brought in by these distantly related sources.

Genetic issues

With each backcross, the progeny becomes more like the recurrent parent. In theory, the BC_4 genotype will be 93.75% identical to the recurrent parent. The mathematical relationship for the recovery of the recurrent parent is presented by W. Allard as $1 - (\frac{1}{2}^{m-1})$, where m is the number of generations of selfing or backcrosses. In another way, the proportion of the donor genes is reduced by 50% following each generation of backcrossing. This is obtained by the relationship $\frac{1}{2}^{m+1}$, where m is the number of crosses and backcrosses to the parent. For example, in the BC_4, the value is $\frac{1}{2}^5 = 3.125\%$. To obtain the percentage of homozygotes for alleles of recurrent parents in any generation, the mathematical relationship is:

$$[(2^{m-1})/2^m]^n$$

where n = number of genes.

Because of cytoplasmic inheritance, it is sometimes critical which of the two parents is used as the female. For example, to use CMS in breeding, the male-fertile inbred lines with normal cytoplasm and non-restorer genes (B-lines) are converted to sterile cytoplasm (A-lines) to be used as male-sterile female lines in a cross.

The resulting cultivar from a backcross breeding program could differ from the starting cultivar beyond the transferred gene(s) because of **linkage drag** from the association of undesirable traits with the genes from the donor. Backcrossing is more effective in breaking linkages over selfing, especially where heritability is low for the undesirable trait.

A certain number of individuals are needed for a chance to recover the desired genes in a backcross program. This number increases as the number of genes controlling the donor trait increases. Furthermore, for multiple gene traits, it will be necessary to grow backcross progeny through to F_2 or later generations to obtain the desired genotypes for advancing the program. When the trait is governed by a dominant gene, it is easy to identify plants carrying the desired gene. However, when the desired trait is conditioned by a recessive gene, an additional step is needed after each backcross to produce an F_2 generation in order to identify the recessive trait. The genetic advance in backcross breeding depends on several factors:

1 **Heritability of the trait**. As previously indicated, traits that are conditioned by major genes and have high heritability are easier to transfer by backcrossing.
2 **Sustainable intensity of trait expression**. Progress with selection will be steadier where the expression of the trait of interest remains at a high intensity throughout the program (i.e., no modifier gene action).
3 **Availability of selection aids**. The ability to identify and select desirable genotypes after the backcross is critical to the success of the procedure. Depending on the trait, special selection techniques may be needed. For disease-resistance breeding, artificial disease epiphytotics may be necessary. Molecular markers may be helpful in selection to reduce the number of backcrosses needed for the program.
4 **Number of backcrosses of the marker**. The genetic distance between the parents is important to the progress made in backcrossing. If both are closely related cultivars, fewer backcrosses will be needed than if the gene transfer is from a wild genotype to an adapted one.

Advantages and disadvantages

The major advantages and limitations of backcross breeding include the following.

Advantages

1 The method reduces the number of field testings needed since the new cultivar will be adapted to the same area as the original cultivar (especially true when both parents are adapted).
2 Backcross breeding is repeatable. If the same parents are used, the same backcrossed cultivar can be recovered.
3 It is a conservative method that does not permit new recombination to occur.
4 It is useful for introgressing specific genes from wide crosses.
5 It is applicable to breeding both self-pollinated and cross-pollinated species.

Disadvantages

1. Backcrossing is not effective for transferring quantitative traits. The trait should be highly heritable and readily identifiable in each generation.
2. The presence of undesirable linkages may prevent the cultivar being improved from attaining the performance of the original recurrent parent.
3. Recessive traits are more time-consuming to transfer.

Modifications

When transferring a recessive gene (*rr*) the backcross will segregate for both homozygous dominant and heterozygous genotypes (e.g., *RR* and *Rr*). To identify the appropriate genotype to advance, it will be necessary to self the backcross to distinguish the two segregants for the *Rr*. Alternatively, both segregants may be used in the next cross, followed by selfing. The backcross progenies from the plants that produce homozygous (*rr*) segregates are heterozygous and are kept while the others are discarded. This is actually not a modification *per se*, since it is the way to transfer a recessive allele.

If a breeding program is designed to transfer genes for multiple traits, it will be more efficient to conduct separate backcross programs for each trait. The backcross-derived lines are then used as parents in a cross to develop one line that contains the multiple traits.

Special backcross procedures

Congruency backcross

The **congruency backcross** technique is a modification of the standard backcross procedure whereby multiple backcrosses, alternating between the two parents in the cross (instead of restricted to the recurrent parent), are used. The technique has been used to overcome the interspecific hybridization barrier of hybrid sterility, genotypic incompatibility, and embryo abortion that occurs in simple interspecific crosses. The crosses and their genetic contribution are demonstrated in Table 16.1.

Table 16.1 The concept of congruency backcrossing.

Cross	Hybrid type	Genetic constitution A : B
$A \times B$	F_1	50 : 50
$F_1 \times A$	BC_1	75 : 25
$BC_1 \times B$	CBC_2	37.5 : 62.5
$CBC_2 \times A$	CBC_3	68.8 : 31.3
$CBC_3 \times B$	CBC_4	34.4 : 65.6
$CBC_4 \times A$	CBC_5	67.2 : 32.8

Advanced backcross QTLs

The **advanced backcross quantitative trait loci** (QTLs) method developed by S. D. Tanksley and J. C. Nelson allows breeders to combine backcrossing with mapping to transfer genes for QTLs from unadapted germplasm into an adapted cultivar. This method was developed for the simultaneous discovery and transfer of desirable QTLs from unadapted germplasm into elite lines. It was briefly discussed in Chapter 14.

Multiline breeding and cultivar blends

N. F. Jensen is credited with first using this breeding method in oat breeding in 1952 to achieve a more lasting form of disease resistance. Multilines are generally more expensive to produce than developing a synthetic cultivar, because each component line must be developed by a separate backcross.

Key features

The key feature of a multiline cultivar is disease protection. Technically, a **multiline** or **blend** is a planned seed mixture of cultivars or lines (multiple pure lines) such that each component constitutes at least 5% of the whole mixture. The pure lines are phenotypically uniform for morphological and other traits of agronomic importance (e.g., height, maturity, photoperiod), in addition to genetic resistance for a specific disease. The component lines are grown separately, followed by compositing in a predetermined ratio. Even though the term multiline is often used interchangeably with blend, sometimes the former is limited to mixtures involving **isolines** or near **isogenic lines** (lines that are genetically identical except for the alleles at one locus). The purpose of mixing different genotypes is to increase heterogeneity in the cultivars of self-pollinated species. This strategy would decrease the risk of total crop loss from the infection of one race of the pathogen, or some other biotic or abiotic factor. The component genotypes are designed to respond to different versions or degrees of an environmental stress factor (e.g., different races of a pathogen).

Applications

One of the earliest applications of multilines was for breeding "variable cultivars" to reduce the risk of loss to pests that have multiple races, and whose incidence is erratic from season to season. Planting a heterogeneous mixture can physically impede the spread of disease in the field as resistant and susceptible genotypes intermingle.

Mixtures may be composited to provide stable performance in the face of variable environments. Mixtures and blends are common in the turfgrass industry. Prescribing plants for conditions that are not clear-cut is challenging. Using mixtures or blends will increase the chance that at least one of the component genotypes will match the environment.

In backcross breeding, the deficiency in a high-yielding and most desirable cultivar is remedied by gene substitution from a donor. Similarly, the deficiency of an adapted and desirable cultivar may be overcome by mixing it with another cultivar that may not be as productive but has the trait that is missing in the desirable cultivar. Even though this strategy will result in lower yield per unit area in favorable conditions, the yield will be higher than it would be under adverse conditions if only a pure adapted cultivar was planted.

Multilines composited for disease resistance are most effective against airborne pathogens with physiological races that are explosive in reproduction. An advantage of blends and mixtures that is not directly related to plant breeding, is marketing. Provided a label "variety not stated" is attached to the seed bag, blends of two or more cultivars can be sold under various brand names, even if they have identical composition.

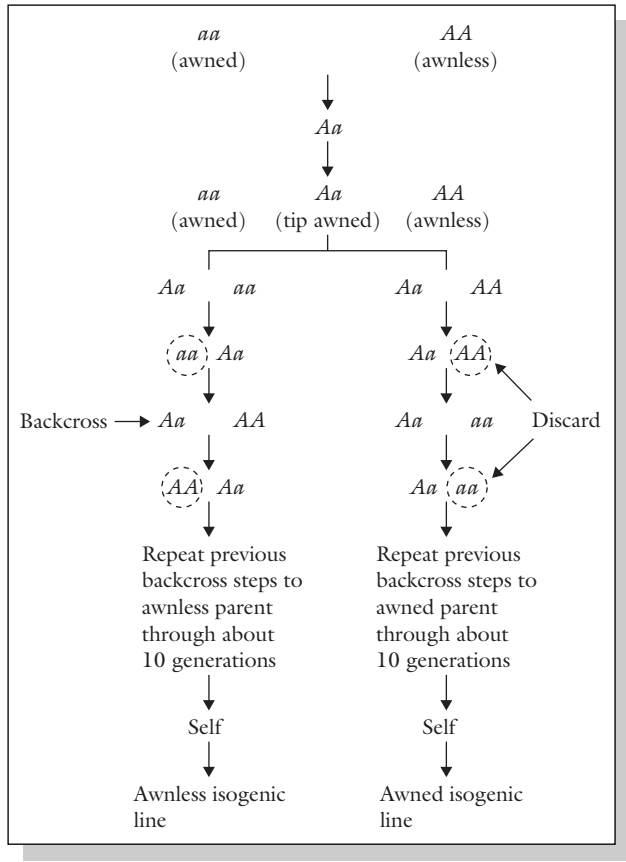

Figure 16.8 Generalized steps in breeding multiline cultivars.

Procedure

The backcross is the breeding method for developing multilines. The agronomically superior line is the recurrent parent, while the source of disease resistance constitutes the donor parent. To develop multilines by isolines, the first step is to derive a series of backcross-derived isolines or near-isogenic lines (since true isolines are illusive because of linkage between genes of interest and other genes influencing other traits). A method for developing multilines is illustrated in Figure 16.8. The results of the procedure are two cultivars that contrast only in a specific feature. For disease resistance, each isoline should contribute resistance to a different physiological race (or group of races) of the disease.

The component lines of multilines are screened for disease resistance at multilocations. The breeder then selects resistant lines that are phenotypically uniform for selected traits of importance to the crop cultivar. The selected components are also evaluated for performance (yield ability), quality, and competing ability. Mixtures are composited annually based on disease patterns. It is suggested that at least 60% of the mixture comprises isolines resistant to the prevalent disease races at the time. The proportion of the component lines are determined by taking into account the seed analysis (germination percentage, viability).

Genetic issues

Multiline cultivars consist of one genetic background but different genes for the trait of interest. A multiline is hence spatially differentiated, plant to plant. When planted, the cultivar creates a mosaic of genotypes in the field to provide a buffering against the rapid development of disease.

Two basic mechanisms are used by multiline cultivars to control disease – stabilization of the patterns of virulence genes and population resistance (see Chapter 20). By stabilizing the patterns of virulence genes in the pathogen, it is supposed that genes for resistance would retain their value in protecting the cultivar for an extended period. The concept of population resistance is the delay in the buildup of the pathogen in the multiline cultivar.

Spore trapping has also been proposed to explain disease buildup in the population of a multiline by reducing the effective inoculation load in each generation. Following the primary inoculation (the initial spores to infect the field), spores that land on resistant genotypes will not germinate. Similarly, progeny spores from susceptible genotypes landing on resistant genotypes will not germinate. The sum effect of these events is a reduction in the inoculum load in each generation.

Advantages and disadvantages

Multilines have certain key advantages and disadvantages.

Advantages

1. A multiline provides protection to a broad spectrum of races of a disease-producing pathogen.
2. The cultivar is phenotypically uniform.
3. Multilines provide greater yield stability.
4. A multiline can be readily modified (reconstituted) by replacing a component line that becomes susceptible to the pathogen, with a new disease-resistant line.

Disadvantages

1. It takes a long time to develop all the isolines to be used in a multiline, making it laborious and expensive to produce.
2. Multilines are most effective in areas where there is a specialized disease pathogen that causes frequent severe damage to plants.
3. Maintaining the isoline is labor intensive.

Modifications

Cultivars can be created with different genetic backgrounds (instead of one genetic background). When different genetic lines (e.g., two or more cultivars) are combined, the mixture is a **composite** called a **variety blend**. Blends are less uniform in appearance than a pure-line cultivar. They provide a buffering effect against genotype × environment interactions.

Composites

As previously stated, a **composite cultivar**, like a multiline, is a mixture of different genotypes. The difference between the two lies primarily in the genetic distance between the components of the mixture. Whereas a multiline is constituted of closely related lines (isolines), a composite may consist of inbred lines, all types of hybrids, populations, and other less similar genotypes. However, the components are selected to have common characters, such as a similar growth period, or degrees of resistance to lodging or to a pathogenic agent. This consideration is critical to having uniformity in the cultivar.

A composite cultivar should be distinguished from a composite cross that is used to generate multiple-parent crosses by successively crossing parents (i.e., single, double, cross, etc.) until the final parent contains all parents. Composites may serve as a continuous source of new entries for a breeding nursery. Any number of entries may be included in a composite, provided selection is judiciously made after evaluation. New entries may be added at any time. Technically, a composite may derive from a single diverse variety, a progeny from a single cross, or even several hundreds of entries. However, a good number of entries lies between 10 and 20. The breeder's objectives determine the kind of entries used for breeding a composite. Using elite and similar genotypes would make the composite more uniform, robust (at least initially), but less genetically diverse. The reverse would be true if diverse entries are included. As a population improvement product, the yield of a composite can be improved by advancing it through several cycles of selection.

In species such as sorghum, which are predominantly self-pollinated, a recessive male-sterility gene that is stable across environments may be incorporated into the composite (e.g., the ms_3 in sorghum) by crossing each entry to the source of the sterility gene prior to mixing. The F_1 (fertile) is first selfed and then backcrossed to the male-sterile segregates. The recurrent parents are then mixed to create the composite.

Recurrent selection

Recurrent selection is a cyclical improvement technique aimed at gradually concentrating desirable alleles in a population. It is one of the oldest techniques of plant breeding. The name was coined by F. H. Hull in 1945. It was first developed for improving cross-pollinated

species (maize) and has been a major breeding method for this group of plants. Hence detailed discussion of this method of breeding is deferred to Chapter 17. It is increasingly becoming a method of improving self-pollinated species. It has the advantage of providing additional opportunities for genetic recombination through repeated intermating after the first cross, something not available with pedigree selection. It is effective for improving quantitative traits.

Comments

1. Recurrent selection requires extensive crossing, which is a challenge in autogamous species. To overcome this problem, a male-sterility system may be incorporated into the breeding program. With male sterility, natural crossing by wind and/or insects will eliminate the need for hand pollination.
2. Adequate seed may be obtained by crossing under a controlled environment (greenhouse) where the crossing period can be extended.

Advantages and disadvantages

There several advantages and disadvantages of the application of recurrent selection to breeding autogamous species.

Advantages

1. Opportunities to break linkage blocks exist because of repeated intercrossing.
2. It is applicable to both autogamous grasses (monocots) and legumes (dicots).

Disadvantages

1. Extensive crossing is required, something that is a challenge in autogamous species. A male-sterility system may be used to facilitate this process.
2. Sufficient seed may not be available after intercrossing. This also may be resolved by including male sterility in the breeding program.
3. More intermatings may prolong the duration of the breeding program.
4. There is also the possibility of breaking desirable linkages.

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Outcomes assessment

Part A

Please answer the following questions true or false:

1. The adapted and highly desirable parent in a backcross is the donor parent.
2. With each backcross, the progeny becomes more like the donor parent.
3. Isogenic lines differ in alleles at a specified locus.
4. A composite may consist of hybrid cultivars.
5. The donor parent is used only once in a cross in a backcross program.
6. Single-seed descent is the oldest plant breeding method.
7. Record keeping is a critical part of the pedigree selection method of breeding.
8. Nilsson-Ehle developed the mass selection procedure.
9. Bulk population breeding is suited to breeding plants that are closely spaced in commercial planting.
10. Mass selection is most effective if the trait of interest has high heritability.

Part B

Please answer the following questions:

1. …….. is the adapted parent in a backcross.
2. Give a specific advantage of multiline cultivars.
3. Give a specific disadvantage of the backcross breeding method.
4. …… developed the pure-line method of plant breeding.
5. Discuss a specific genetic issue involved with mass selection.
6. Give a specific disadvantage of the pedigree method of breeding.

Part C

Please write a brief essay on each of the following topics:

1. Discuss the key features of backcross breeding.
2. Discuss the application of multiline breeding.
3. Distinguish between composite breeding and multiline breeding.
4. Discuss the advantages of bulk breeding.
5. Discuss the advantages of the single-seed descent method of selection.
6. Compare and contrast the mass selection and pure-line selection methods of breeding.
7. Describe the steps involved in the mass selection method of breeding.