

CHAPTER 4

PLANT BREEDING David Lockett and Gerald Halloran

WHAT IS PLANT BREEDING AND WHY DO IT?

Plant breeding, or crop genetic improvement, is the production of new, improved crop varieties for use by farmers. The new variety may have higher yield, improved grain quality, increased disease resistance, or be less prone to lodging. Ideally, it will have a new combination of attributes which are significantly better than the varieties already available. The new variety will be a new combination of genes which the plant breeder has put together from those available in the gene pool of that species. It may contain only genes already existing in other varieties of the same crop, or it may contain genes from other distant plant relatives, or genes from unrelated organisms inserted by biotechnological means.

The breeder will have employed a range of techniques to produce the new variety. The new gene combination will have been chosen after the breeder first created, and then eliminated, thousands of others of poorer performance. This chapter is concerned with describing some of the more important genetic principles that define how plant breeding occurs and the techniques breeders use.

Plant breeding is time-consuming and costly. It typically takes more than ten years for a variety to proceed from the initial breeding stages through to commercial release. An established breeding program with clear aims and reasonable resources will produce a new variety regularly, every couple of years or so. Each variety will be an incremental improvement upon older varieties or may, in rarer circumstances, be a quantum improvement due to some novel gene, the use of some new technique or a response to a new pest or disease. In most of the field crops where considerable genetic improvement has already occurred (*e.g.* wheat, barley, maize, cotton) most new varieties exhibit an improvement of 5% or less over the nearest commercial rival.

In Australian agriculture new plant varieties are most commonly encountered in the major crop species. However, the same principles apply to all other horticultural crops, tree and pasture species. In perennial species the individual plants may take many years to reach maturity before the value of the new gene combination can be assessed. This only serves to lengthen the breeding program and provide greater challenges for the breeder.

The advantage of a new plant variety may be specific to certain growing areas and conditions, or it may have attributes that are not required in other regions. Bread wheat varieties for South Australia require, among many other characters, boron tolerance and resistance to cereal cyst nematode (CCN) because many soils in the region have high boron levels, and CCN populations can cause significant yield losses. In New South Wales the edaphic and biotic stresses are different – new wheat varieties

must have acid-soil tolerance and resistance to the fungal disease *Septoria triticii* blotch.

As the list of requirements for any new variety increases, the breeding program must handle more and more material to have a reasonable chance of isolating a new, improved gene combination (genotype). For a given level of resources (labour, land, operating funds) a program will have an upper size limit depending on the species involved and the aims of the program. Consequently, there are often several breeding programs for a crop in a single country. The programs may compete head-to-head for market share, or may target different niche markets, especially if they are funded by an organisation with some commercial interest in that market.

A breeder will typically collaborate closely with plant pathologists, entomologists, biochemists, agronomists, seed production professionals, molecular biologists, statisticians, and computer scientists. An efficient and productive breeding program will draw on these disciplines and use the latest proven technology. Breeding programs in the developed world are highly mechanised and employ the latest bulk-handling techniques (Figure 4.1).

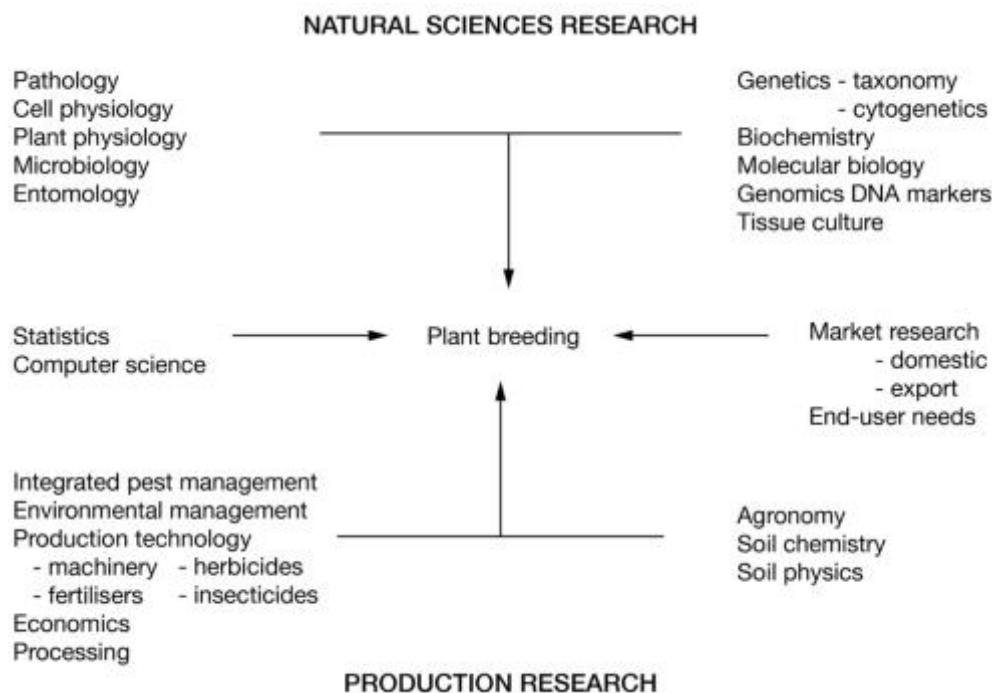


Figure 4.1 The central nature of plant breeding

The world's population is increasing at an alarming rate. The land area available for farming is decreasing due to urbanisation, and increasing salinity, acidity and soil erosion. The terms of trade in agricultural commodities have been declining steadily for over 50 years. Pests and diseases of our crop plants are continually evolving to overcome the resistance that exists in current varieties. Plant breeding is therefore a race to synthesise or construct new genotypes to maintain or increase production per unit area of land with reduced inputs and maximised quality of product. From an economic point of view, the adoption of a new plant variety is cost-neutral to the

farmer (aside from seed costs). Rigorous economic analysis has shown that public investment in plant breeding pays high returns and this is why all successful crop industries are under-pinned by adequately resourced breeding programs.

To save money and deliver varieties as quickly as possible, breeders use a range of techniques to speed up the process. Off-season nurseries, often on the other side of the globe, may be used (if there are no quarantine restrictions) to grow two generations per year. The early generations of the program may be grown in the glasshouse or growth-room to achieve pure-breeding genotypes as soon as possible.

Any new plant variety is always going to be part of a farming system and will only achieve its genetic potential if the agronomy and other farming technology is in place. Other chapters consider these issues.

Plant breeding consists of four main phases all of which run concurrently in an established program:

Phase 1 The breeder identifies the needs of the farmers and the deficiencies in the current varieties. Perhaps improved or new disease resistance is required, or increased seed size, or simply increased yield to make the crop profitable. The breeder will then collect together the separate genotypes that have the attributes required. This may require screening the available germplasm collections (see below) or obtaining from other breeders genotypes described in the scientific literature. If the attribute required is not available in the species gene pool then the breeder may consider using gene technology to obtain the gene from elsewhere or, as a last resort, consider synthesising a completely new gene. These latter two options will be long-term strategic decisions depending on the value of overcoming the deficiency and the facilities and collaborators available.

Phase 2 The second phase of the breeding program is to artificially hybridise (or 'cross') the identified parents to bring the genes for the desirable attributes together in the same hybrid individual. The precise procedures will depend on the species involved, and any pre-existing knowledge of the genetic control of the attributes

Phase 3 In the early, segregating generations the breeder selects the progeny of the crosses so as to remove those with undesirable or inferior genotypes, progressively moving towards a smaller number of elite lines. This third phase is the largest part of a breeding program and involves identifying the products of genetic segregation and recombination and finding the 'best of the bunch' as reliably and as quickly as possible, while minimising the risk of failing to retain a superior line. Various selection procedures are used by breeders (see below).

Phase 4 The final breeding phase consists of establishing the worth of any new genotype over the existing varieties, bulking up sufficient quality seed for distribution to farmers and, finally, release of the new variety. The last phase also consumes significant breeding resources since, although only a small number of advanced lines remain in the program each year, they have to be evaluated in an extensive field trial program at many locations, and large seed quantities produced. Breeders constantly

face the dilemma of having varieties released as quickly as possible while still having reliable data on a variety's regional performance and its likely performance in a range of years. Breeders are acutely aware that a farmer needs convincing data in order to change variety and feel a strong responsibility to provide information that is as accurate and as complete as possible.

The reader is referred to basic genetics texts to explain the terms used here since a complete coverage of genetics in this context is not possible (*e.g.* Appels *et al.*, 1998).

CROP PLANT DOMESTICATION

Prior to the beginnings of agriculture, humans were nomadic food gatherers. They ate fruits and berries and the seeds of grasses and of a range of dicotyledonous species. Archaeological evidence indicates that among the plants supplying them with seed were the ancestors of present-day crop plants and, in addition, many of the present weeds of agriculture. With increasing demands on food supply, due most likely to population increases, our forebears were forced gradually to adopt a system of deliberate sowing and harvesting of food plants. In areas where rainfall was limiting, they also developed irrigation.

During domestication varieties were selected for those species which were consistently productive, whose seed could be stored, and which were able to provide people with a food that satisfied both their nutritional requirements and their qualitative preferences for such characteristics as taste, colour and texture. These were the species that have become the crops of present-day agriculture and horticulture. Those species not chosen or retained but which were able to adapt to the changed conditions imposed by human farming activities became the weeds of agriculture.

Some of these weeds are close relatives, and even ancestors, of crop plants. Throughout the history of domestication, many exchanged genes with the crop plant through repeated hybridisation with it. In fields of cultivated rice (*Oryza sativa*) in south-east Asia, for example, two weed species, *O. rufipogon*, an annual, and *O. nivara*, a perennial, occur and hybridise with the cultivated species. Similarly, in and around fields of cultivated maize (*Zea mays*) in Central America, two weed species, teosinte (*Zea mexicana*) and *Tripsacum* are still to be found. These species have contributed substantially to genetic changes in maize under domestication through repeated hybridisation with it (Mangelsdorf, 1965).

Most present-day crop plants have had long histories of domestication during which time, in conjunction with increased productivity, marked changes have taken place in many morphological and physiological characters. These changes have been in the direction of increased seed size and number and the reduction or loss of adaptive characters of seed, such as dormancy and dispersal mechanisms. A significant difference between the wild and weed relatives and cultivated forms of wheat and barley, for example, is the change from the fragile to the non-fragile rachis. This change has occurred under domestication and has been most likely strongly selected for by humans. Genetic changes as a result of domestication and agriculture have rendered

most crop plants unable to survive in the wild. Not only have humans been dependent on the crop for their survival, but the crop has become dependant on humans for its continued existence.

The adoption of plant species into agriculture took place in the areas of natural distribution of the wild forms. Many of these wild and subsequent weed forms are still in existence today and knowledge of their geographical distributions has been used to indicate the likely geographical origins of present-day crop species. Vavilov (1926) described the concepts of primary and secondary centres of origin of crop plants. A primary centre of origin was defined as an area of the world where the wild relatives of the crop plant occurred together with the domesticated forms, in what appeared to be the area of original distribution of wild species. An example is the restriction of the distribution of wild diploid wheat, *Triticum monococcum*, and wild tetraploid wheat *Triticum dicoccoides* to the Fertile Crescent (Northern Iraq, Southeastern Turkey, Lebanon and Israel), indicating that this was the likely area where these wheat forms were first domesticated. A secondary centre of origin, or diversity, was regarded as an area where the crop plant occurred in a wide range of types but without the wild forms. It was likely to have been taken from the primary centre early in the history of cultivation of the crop. Vavilov proposed eight primary centres of origin of crop plants, six of them in the Old World and two in the New World. Examples of some of the cultivated plant species occurring there are shown in Table 4.1. While these concepts proposed by Vavilov have had a profound impact on evolutionary studies of crop plants and plant collecting activities, it is now believed that plant domestication was much more complex in evolutionary terms and of wider geographical extent than previously believed. Some crops originated in more than once centre and with some crops that area of origin is not known. Others appear to have been domesticated over vast areas and not in more restricted geographical areas as the centres-of-origin theory of Vavilov implied. Harlan (1976) proposed the idea of diffuse origins of many crop plants. The areas of origin proposed by him for most crop plants are geographically less localised than the areas proposed by Vavilov.

Table 4.1 Centres of original domestication of some important crop plants as proposed by Vavilov (1926)

Centre	Species
1. Chinese	Soybean, apricot, peach, orange
2. Indian	Rice, chickpea, cucumber
2a. Indo-Malayan	Banana, coconut
3. Central Asiatic (Afghanistan, Tibet, Iran)	Bread wheat, cereal rye, peas, pear, apple, walnut
4. Near Eastern (Transcaucasia, Turkey, Syria, Southern Russia)	Diploid wheat, barley, lucerne
5. Mediterranean	Durum wheat, oats, broad bean, lettuce, cabbage, olive
6. Abyssinian	Durum wheat, barley, peas, flax
7. South and Central American	Maize, common bean, pepper, cotton (upland), squash, pumpkin
8. South American (Peru, Ecuador, Bolivia)	Sweet potato, potato, Lima bean, tobacco, tomato
8a. Chile	Potato
8b. Brazilian-Paraguayan	Potato

Knowledge of the geographical origins and evolution of crop plants and their wild and weed relatives is fundamental for genetic and evolutionary study, and for conserving a wide range of genetic variability for crop breeding needs (Simmonds, 1976).

Gene Pools

Present-day crop breeding draws on useful genetic variability, not only within the crop species but also from related species and genera. An example of the use of distantly related genetic variability in crop breeding is the incorporation in wheat of a gene for resistance to stem rust (*Puccinia graminis tritici*) from *Agropyron elongatum* (Knott, 1963). This species is a distant relative of wheat but it is able to be hybridised with wheat and possesses genes that can usefully contribute to the genetic improvement of the crop species. The concept of the gene pool of the species embraces genetic variability within the cultivated forms, its wild and weed relatives and more distantly related species which can be hybridised with the commercial species and can contribute genes for its improvement. Harlan *et al.* (1973) recognised the gene pool of a species as having primary and secondary levels related to the degree of accessibility of genetic variability for crop breeding within closely and more distantly related species. This involves considerations such as cross-compatibility, hybrid viability and chromosome pairing in hybrids with the crop species.

Modern techniques in genetic engineering or molecular biology have greatly extended the concept of the gene pool to include any gene in existence (in any species) or any gene that can be designed and constructed in the laboratory. Alien or novel genes used in plant breeding bring with them potentially huge benefits but also a range of fundamental difficulties: those of integration, reliable inheritance, and expression. However, these technical difficulties are likely to be overcome in time and transgenic varieties will increasingly be considered a normal part of the potential gene pool. Many people are ethically and morally opposed to transgenic organisms and the probability of their wide acceptance by consumers remains unclear.

Genetic Conservation

The life blood of crop breeding for further improvements in yield, disease resistance, quality and other characters is the genetic variability available within the gene pool of the species. Therefore, to retain maximum flexibility in breeding and the ability to respond to new threats and challenges to the crop, it is necessary to have available as wide a range of genetic variability as possible. Since many of the long-term future crop breeding needs are unknown it is necessary that genetic variability, regardless of its immediate value, be conserved for future crop improvement.

Most of the original genetic variability of crops was located in areas of primitive agriculture. The implementation of so-called agricultural improvement and development programs in these areas has caused much of the variability of the primitive varieties and associated weed species to be lost due to the widespread cultivation of new, uniform varieties often from overseas.

Breeders and geneticists are keenly aware of the need to conserve genetic variability for future crop breeding (Frankel and Hawkes, 1975). The Food and Agriculture Organisation (FAO) of the United Nations funds much of the collection and maintenance of genetic variability of crop plants and related species, along with the international breeding centres in the CGIAR network.

A number of countries maintain gene banks (or germplasm collections) of many of the world's crop plants. These are large collections of advanced varieties and crossbreds, primitive landraces, wild and weed relatives, and other related species of the crop plant. The United States Department of Agriculture maintains a large gene bank for crop plants at Beltsville, Maryland, and Russia has a similar collection at the N.I. Vavilov All-Union Institute of Plant Breeding at St Petersburg. In Australia a national winter cereals collection has been established at Tamworth, New South Wales, for use by Australian breeders. Germplasm collections of other crop plants and their wild relatives important to Australian agriculture are held at CSIRO in Canberra, Biloela in Queensland, Perth in Western Australia, and Horsham in Victoria.

In the gene bank, storage is mainly as seed – kept under conditions of low humidity and low temperature to ensure its viability for 10 to 20 years. Interest has been shown in the possibility of storing pollen of crop plants in gene banks but it generally has a much shorter viability than stored seed. Pollen collections are also much more difficult to regenerate than seed collections, a factor which is a serious limitation to the facility of maintaining the viability of lines in a gene bank. Before the viability declines to a low level, the seed collections are sown and harvested to produce fresh seed and in this way the gene bank can be perpetuated. Some of these collections are being extended to accommodate vegetative propagules (such as tissue or single-cell cultures) of those species whose seed or pollen is not easily stored due to rapid loss of viability.

During Phase 1 of a breeding program the breeder electronically searches the germplasm collections of the species concerned for accessions that might contain the genes required. Seed samples can then be procured to start the crossing program (Phase 2).

GENETICS AND CROP BREEDING

Present-day crop breeding and selection methods are based on the genetic principles propounded in Mendel's laws of inheritance. Many characters of crop plants exhibit simple inheritance; that is, their expression is controlled by one gene, or a small number of genes, of large effect whose expression is little influenced by environment. They are called major genes and can be bred and selected for on the basis of segregation frequencies expected from simple Mendelian inheritance. Other characters owe their expression to a large number of genes, each of small effect. These characters are said to exhibit quantitative inheritance and the field of their genetic study and selection is called quantitative genetics. A number of characters exhibit a combination of both types of genetic control; major genes determine wide differences in their expression, and genes of smaller effect have an influence within, and sometimes beyond, the range of the major genes.

Simple Inheritance

Characters of simple inheritance in crop plants segregate similarly to those obtained by Mendel for seed shape and flower colour in peas and can be bred and selected for with relative ease. A character under simple genetic control segregates into discrete classes which can be visually assessed. It is referred to as a qualitative character. Such characters include many forms of disease resistance, morphological and colour variation of the plant or seed and, in some crops, flowering time and its component processes. However, segregation ratios for some characters under the control of a small number of major genes quite often depart from simple Mendelian inheritance. This can be due to interactions between major genes if at least two genes are involved, linkage with other genes, alteration of their expressions by modifier genes, or environmental influences.

Quantitative Inheritance

Many important characters of crop plants exhibit inheritance patterns that are not divisible into classes but show a continuous gradation in expression from that of one parent to the other. The character which segregates to give continuous gradation in expression is referred to as a quantitative character and is specified accurately only in terms of measurable quantities such as length, time, weight or proportion. While quantitative characters do not exhibit discrete Mendelian class segregation, the principles of Mendelian genetics, with some elaboration, are still used to explain their inheritance. However, most quantitative characters in crop plants can only be analysed as the combined effect of all the genes governing their expression.

Heritability

The phenotypic expression of most quantitative characters is the consequence of interaction between the genes a plant carries for that character (its genotype) and the environment in which it has been grown. Variation in phenotype caused by different environments can be used to obtain an estimate of the environmental versus the genotypic component of phenotypic expression. Characters, such as yield and grain quality, whose expressions are dependent on highly-variable environmental factors (e.g. moisture, nutrient availability and temperature) have very large environmental components. Consequently these characters are said to have low heritability.

In order to conduct selection effectively as part of a plant breeding program (Phase 3) it is important to be able to partition phenotypic variance for a character into that due to both genetic and environmental causes. The total variability of a population for a particular character is called the phenotypic variance (V_P), which is the summation of the genetic variance (V_G) and the environmental variance (V_E) (Mather and Jinks, 1982; Falconer and Mackay, 1996) such that

$$V_P = V_G + V_E$$

Estimates of these genotypic and environmental components of variability can be obtained by growing the population as families in a number of environments (locations or years, or both). Heritability (H) is the ratio of the genetic variance over the phenotypic variance

$$H = V_G / (V_G + V_E)$$

Environmental influence on gene expression can range from little or no effect to a very pronounced effect that masks the genetic differences between individuals. A representation of the likely effect of different degrees of environmental influence on the second-generation (F_2) segregation patterns of a single gene is given in Figure 4.2. With 100% heritability the F_2 segregation ratios are 1:2:1 (dominant: heterozygote: recessive) or 3:1 (dominant: recessive) according to whether the character exhibits no, or complete, dominance respectively. As heritability decreases to 75%, i.e. as the environmental influence increases, each of the F_2 segregation classes exhibits increased variance. When heritability is only 25%, the segregation of the F_2 population is as one continuous distribution whether or not the gene exhibits complete dominance.

In practice, low heritability of, say, 0.25 means that a relatively large population of progeny from a cross will need to be examined over a longer period to find successfully the best genotypes, and that errors due to environmental influences will occur along the way. With high heritability the desirable genotype will be much more easily identified and with greater reliability.

With additional information it is possible to partition the quantitative genetic variance for a character into smaller components (additive effects, dominance effects and epistasis). With this knowledge the breeder can better design the breeding program. Further information on methods of calculating the components of genetic variance are given in the detailed discussions of this topic in Mather and Jinks (1982) and Falconer and Mackay (1996).

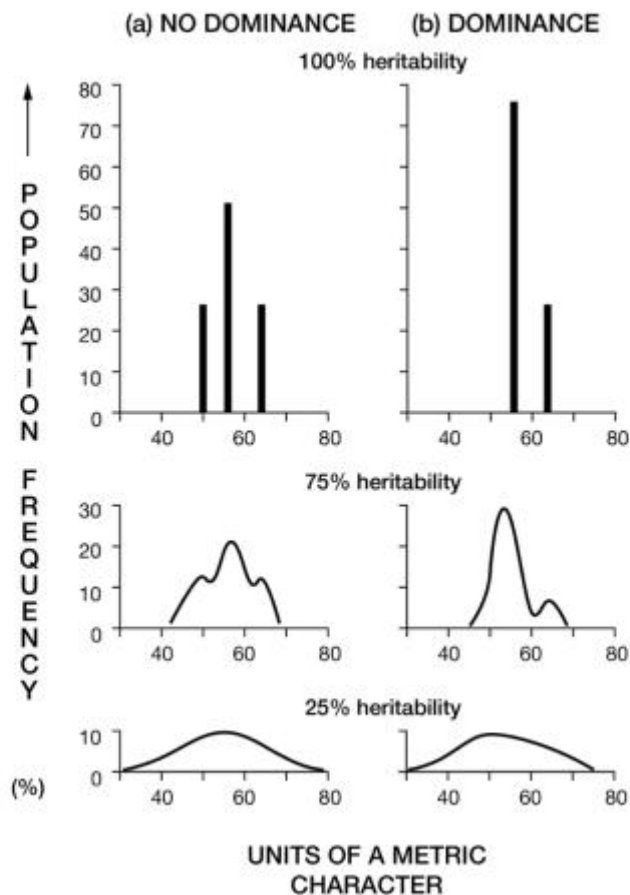


Figure 4.2 F₂ distributions for a monogenic character by which the parents differ by twelve metric units in situations of (a) no dominance and (b) complete dominance with different levels of heritability (100, 75, 50 and 25 per cent) for the character

Linkage

A problem confronting the plant breeder is that of linkage of favourable genes with unfavourable ones in segregating populations. Linkage is when genes are located on the same chromosome arm. Linkage in crop breeding, when one is concerned with improving the genetic performance of highly improved varieties, is a major problem limiting selection advance. It restricts independent assortment of genes, produces an abundance of parental combinations and limits the chance of obtaining the desired recombinants.

An indication of the effect of the degree of linkage on recombination can be gained from considering a theoretical case of linkage in a diploid plant of a dominant gene for resistance (*R*) with an undesirable recessive gene (*l*) conferring lodging susceptibility. Assume that the genotype *RRll* was being used as a parent in a breeding program to incorporate the disease resistance gene, *R*, into a commercial variety of the genotype *rrLL*. The cross would be made between these two parents and the F₁ and F₂ genotypes and phenotypes would be as shown in Figure 4.3. With independent segregation (no linkage) between genes and *R* and *l*, the genotype classes and their proportions in the F₂ would be as shown in the two-way table (Table 4.2). The desired recombinant (*RrLl*) would have a frequency in the F₂ of 1:16 or 6.25%.

However, if the genes *R* and *l* exhibit linkage the recombination fraction of the desired genotype (*RRLL*) in the F_2 would be less than with independent assortment and its frequency will vary according to the degree of linkage, i.e. the closeness of these gene loci on a particular chromosome. The closer they are together the lower will be the recombination frequency, i.e. the lower the chance for a crossover occurring on the chromosome between genes *R* and *l* to separate them. When the two desired genes are present on different homologous chromosomes in the F_1 (as here) they are said to be in repulsion linkage. The influence of the recombination value, or linkage, on the frequency of the recombinant genotype (*RRLL*) in the F_2 is shown in Table 4.2

Table 4.2 Influence of linkage on the expected frequency of genotype *RRLL* in the F_2 from the two types of F_1 double heterozygote

Recombination value (linkage)	Percentage of <i>RRLL</i> genotypes in F_2 if the F_1 is:	
	<i>RL</i> / <i>rL</i> Repulsion	<i>RL</i> / <i>rl</i> Coupling
0.50 (Independent assortment of <i>R</i> and <i>l</i>)	6.25	6.25
0.25	1.56	14.06
0.10	0.25	20.25
0.02	0.01	24.01
0.01	0.0025	24.50
<i>p</i>	$\frac{1}{4} p^2$	$\frac{1}{4} (1-p)^2$

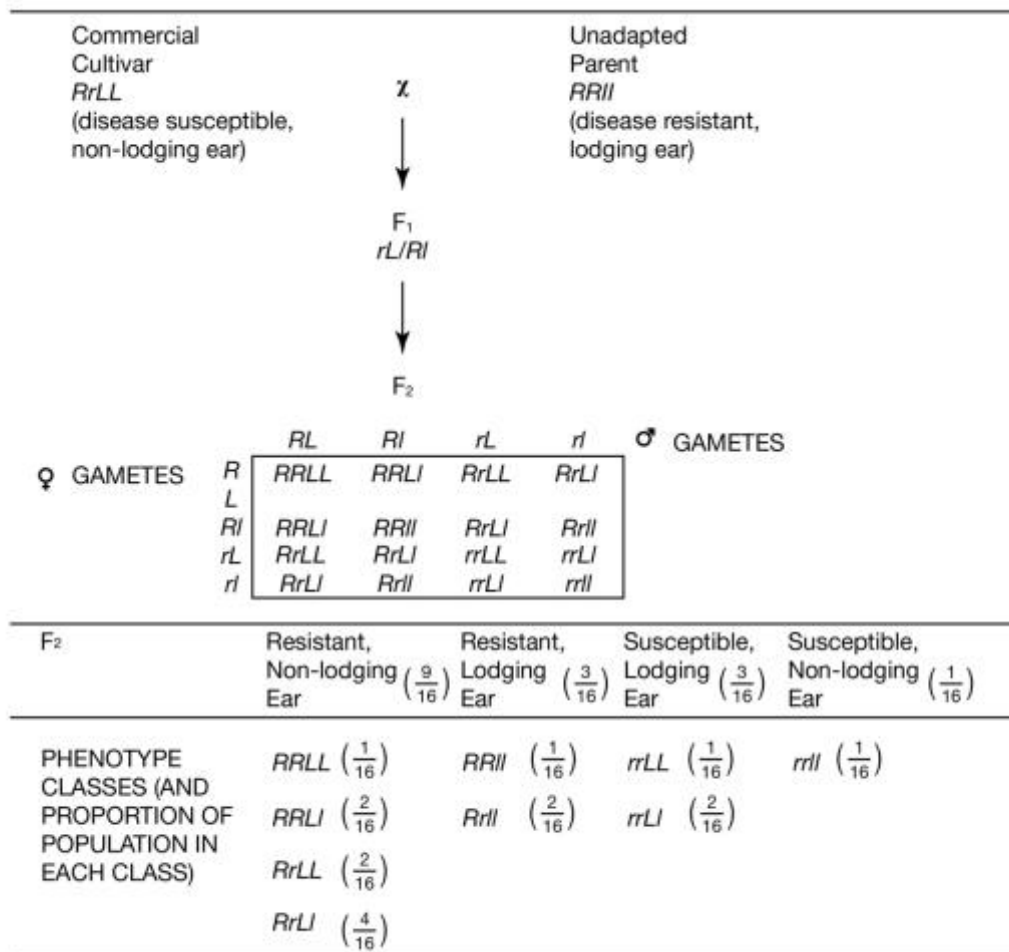


Figure 4.3 Phenotypic and genotypic segregation classes for two genes (disease resistance, *R*, vs susceptibility, *r*, and non-lodging, *L*, vs lodging, *l*, ears), in the F_2 of a wheat cross whose parents differ for these characters

In the converse situation, a cross might be made between two parents, *RRLl* and *rrll* in order to manipulate some other character. In this case, the *RL* gene combination is already present on the same homologous chromosome (coupling linkage). Among the F_2 segregants the *RL* gene combination will tend to be held together and will appear at greater-than-expected frequency depending on the recombination value (Table 4.2). A very useful means of breaking a linkage between desirable and undesirable genes is the backcross method (see below).

Genetic advance under selection

The rate at which the plant breeder can achieve genetic advance in selecting for a particular character will depend on three main factors:

- the amount of genetic variability in the original population for the character being selected must provide adequate potential to achieve the level of improvement envisaged;
- the masking effect of the environment on the genes for the character must not be so high as to limit the variability of its expression for selection, i.e. the character must exhibit a moderately-high level of heritability; and
- closely related to the other two considerations, the intensity of selection must be such that it does not restrict the rate of genetic advance.

Plant breeders are interested in calculating the likely genetic advance they can expect from selection, whether it be a cross-fertilising population, a segregating population, or a set of homozygous lines. Among populations of different genetic origins they are interested in estimating the relative selection advance likely to be obtained from each population.

Consider the use of these predictive estimates for a situation where a breeder wishes to ascertain the likely gains in yield from selection amongst a set of n self-pollinated lines; the n lines are grown in a number of locations in a number of seasons and evaluated for yield of each line (Y). Assuming the values of Y amongst the lines are normally distributed with a standard deviation of σ_Y the frequency distribution for yield will be as shown in Figure 4.4. If the breeder sets the yield selection value at a level Y^1 , the selected families are those falling in the stippled area below the yield distribution curve and represent a proportion (b) of the population. By calculus it can be shown that the expected genetic gain represented by the selected lines is

$$G_s = k \sigma_y H$$

where G_s is the expected genetic gain in yield, σ_y is the standard deviation of the mean yields of the total n lines, H is the heritability and k is the selection differential. The value G_s measures the difference between the mean genotypic value of the l selected lines, a_s and the mean genotypic value of the n original lines, a . Therefore,

$$G_s = a_s - a$$

The selection differential value, k , is assessed from the mean phenotypic value of the l selected lines (Y_s), the mean phenotypic value for the total number (n) of lines tested, the phenotypic standard deviation (σ_Y), and the intensity of selection l/n . The value, k , is expressed in standard deviation units and varies only according to variation in selection intensity. If selection for yield retains 5% of the lines, k can be shown to have a value of 2.06. If 20% of the lines are retained k has a value of 1.40. The formula for calculating the rate of genetic advance under selection evaluates comparatively the three components, genetic variability, environmental effects and selection intensity for the character under study.

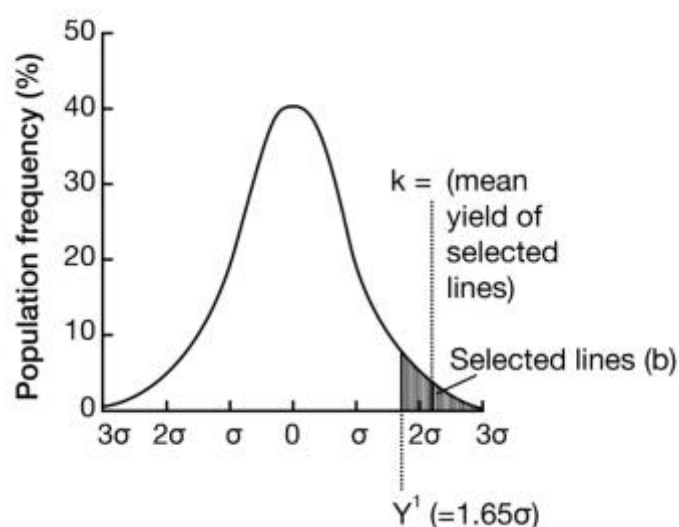


Figure 4.4 Theoretical distribution of mean yields of lines from replicated yield trials. Assuming the breeder selects the upper 5 per cent of lines, i.e. with yields greater than $Y^1 (=1.65\sigma)$, the mean of the b selected lines is expected to be k , the selection differential

REPRODUCTIVE SYSTEMS IN PLANTS

The mode of reproduction (breeding system) of crop plants influences the level of genetic variability present in the crop population and the breeding and selection methods suitable for plant improvement. In addition, the particular type of reproduction can impose practical limits on the efficiency of certain breeding and selection procedures. For this reason breeders have sought to alter genetically the breeding system of crops. The use of cytoplasmic male-sterility and the modification of self-incompatibility are examples of alterations that have been effected in breeding systems to produce new methods for crop improvement. In addition, the breeding system must be manipulated to produce hybrid varieties (see below).

Crop plants can be divided into two classes on the basis of their common modes of reproduction, sexual and asexual. Sexually reproducing species (which includes most of the major crop plants and horticultural species) can be divided into two groups – those that are predominantly self-pollinating and those that are predominantly cross-

pollinating. The modes of reproduction of the most common crop plants are shown in Table 4.3.

Cross-Pollinating Species

Cross-pollination confers on plant populations a far greater degree of genetic variability and flexibility than does self-pollination. Under domestication, inbreeders appear to have arisen from outbreeders, but rarely the reverse. Amongst crop plants the self-pollinating character is much more common than cross-pollination (Table 4.3). The self-pollinating character gives the species a greater immediate fitness than does the cross-pollinating character and therefore may be more favoured under the relatively stable conditions of agriculture.

Table 4.3 Modes of reproduction of the major crop plants

Crop type	Mode of reproduction	
	Self-pollinated ^a	Cross-pollinated
Cereals	Barley	Maize
	Oats	Rye
	Wheat	
	Rice	
	Sorghum	
	Foxtail millet	
Legumes	Broad bean	Scarlet runner bean
	Chickpea	Faba bean
	Cowpea	
	Field pea	
	Lupin	
Oilseeds	Linseed	Safflower
	Canola	Sunflower
Fibre crops	Cotton	Hemp
	Flax	
Other crops	Tobacco	Sugar beet
	Tomato	Sugar cane
		Sweet potato

^a Most self-pollinated crops have been shown to exhibit small amounts of cross-pollination (1 to 10 per cent)

Cross-pollinating species have a number of mechanisms which either minimise the level of selfing or totally preclude it. One such mechanism, *monoecism*, uses the separation of male and female flowers on the plant to increase the chance for cross-pollination. The asynchronous maturation of anthers and stigma of flowers, whether they be monoecious or *hermaphroditic* (i.e. having bisexual flowers), is another method of ensuring mainly cross-pollination. When pollen is shed first in such flowers the mechanism is called *protandry*; when the stigma is receptive first it is called *protogyny*. The combination of monoecism and protandry in maize can ensure almost complete out-crossing. A second mechanism, *dioecism* (i.e. separate male and female individuals) prevents selfing but does not prevent brother-sister mating. It is not common in higher plants and is the breeding system of only a few crop plants such as hemp, hops and jojoba. Thirdly, *self-incompatibility* is much more widespread and is found in most cross-pollinating species.

Self-Incompatibility

Cross-pollinating plant populations have a genetic structure in which the heterozygote is favoured and the individual which arises from selfing is adaptively disadvantaged. The reason for this is believed to be that selfing of heterozygotes produces individuals with many genes which are double recessive, in which condition they are deleterious to normal vigour, growth and fertility. This phenomenon is also known as *inbreeding depression*. Self-incompatibility is an efficient mechanism of either preventing or minimising the amount of selfing in cross-pollinated plants thereby keeping inbreeding to a minimum. The incompatibility reaction is determined in the stigma where mutual recognition between pollen and stigma is necessary for pollen tube growth down the style to effect fertilisation. The recognition is between pollen-wall proteins and glycoproteins interacting with proteins on the surface of the stigma.

Two types of incompatibility are present in cross-pollinating species, gametophytic and sporophytic incompatibility. They are both genetically controlled and in most cases by a single gene which, in some species, has a small number of alleles but in others a very large number.

Gametophytic Incompatibility This system of incompatibility is usually controlled by a single gene, *S*, which has a number of different alleles. Incompatibility is brought about by inhibition, or gross retardation, of the growth of pollen tubes on styles whose tissue contains the same allele of *S*. As a result, plants are always heterozygous at the *S* locus. Gametophytic incompatibility produces three main types of pollination pattern depending on the allelic constitution of the (male) pollen and the (female) style at the *S* locus (Figure 4.5). In the cross where the allelic constitution of the parents at the *S* locus is

female ($S_1 S_2$) x male ($S_1 S_2$)

the stilar tissue, which is diploid, will be $S_1 S_2$. It will inhibit pollen tube growth of both types of haploid pollen combinations, i.e. S_1 and S_2 , and gives 100% incompatibility. In the cross where the parent allelic constitution is

female ($S_1 S_2$) x male ($S_1 S_3$)

the stilar tissue will inhibit only S_1 pollen. S_3 pollen tubes will pass down the style to the ovule and fertilise both S_1 or S_2 eggs. In this cross there will be 50% incompatibility in the pollen-style reaction. In the cross where the allelic constitution of the parents is

female ($S_1 S_2$) x male ($S_3 S_4$)

there will be 100% compatible combinations because the stilar tissue does not possess an allele in common with either of those in the pollen.

Sporophytic Incompatibility This system is similar to the gametophytic system in that control is exercised by a single gene with multiple alleles. However, it differs from the gametophytic system in a number of ways. Firstly, the incompatibility reaction is

determined by the genotype of the plant in which the pollen is borne. Another difference from the gametophytic type of compatibility is that its alleles may exhibit dominance, independent action or competition in either pollen or styles depending on the type of alleles present at the *S* locus. It is genetically much more complex than the gametophytic system and in consequence leads to a larger number of compatibility reactions.

It is possible in the sporophytic system, because of reversal of the order of dominance of alleles in the pollen as against their action in the style, for reciprocal crosses to exhibit complete compatibility and complete incompatibility. For example, if with the three alleles S_1 , S_2 and S_3 the order of dominance in stylar tissue was $S_1 > S_2 > S_3$ but in the pollen reactions $S_3 > S_2 > S_1$, this reversal of dominance would lead to reciprocal differences in compatibility versus incompatibility as shown in the cross $S_1 S_2 \times S_2 S_3$ and its reciprocal (Figure 4.6).

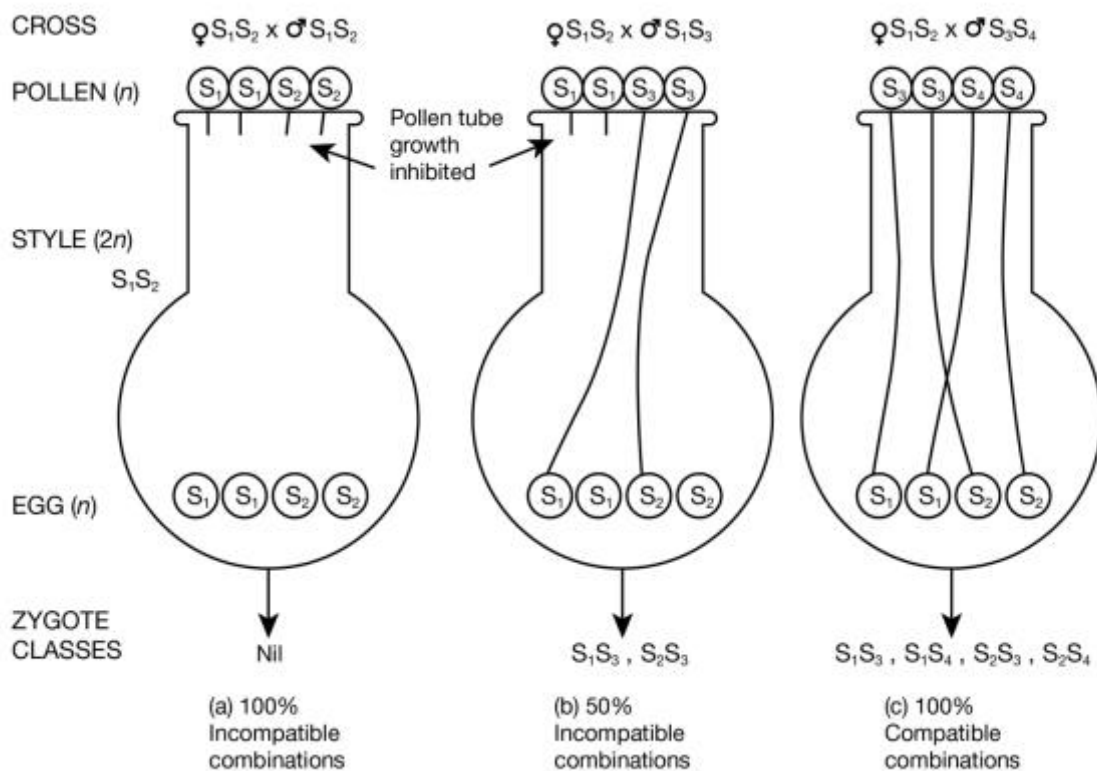


Figure 4.5 Gametophytic incompatibility showing pollen-style interactions according to their genetic constitution for *S* alleles

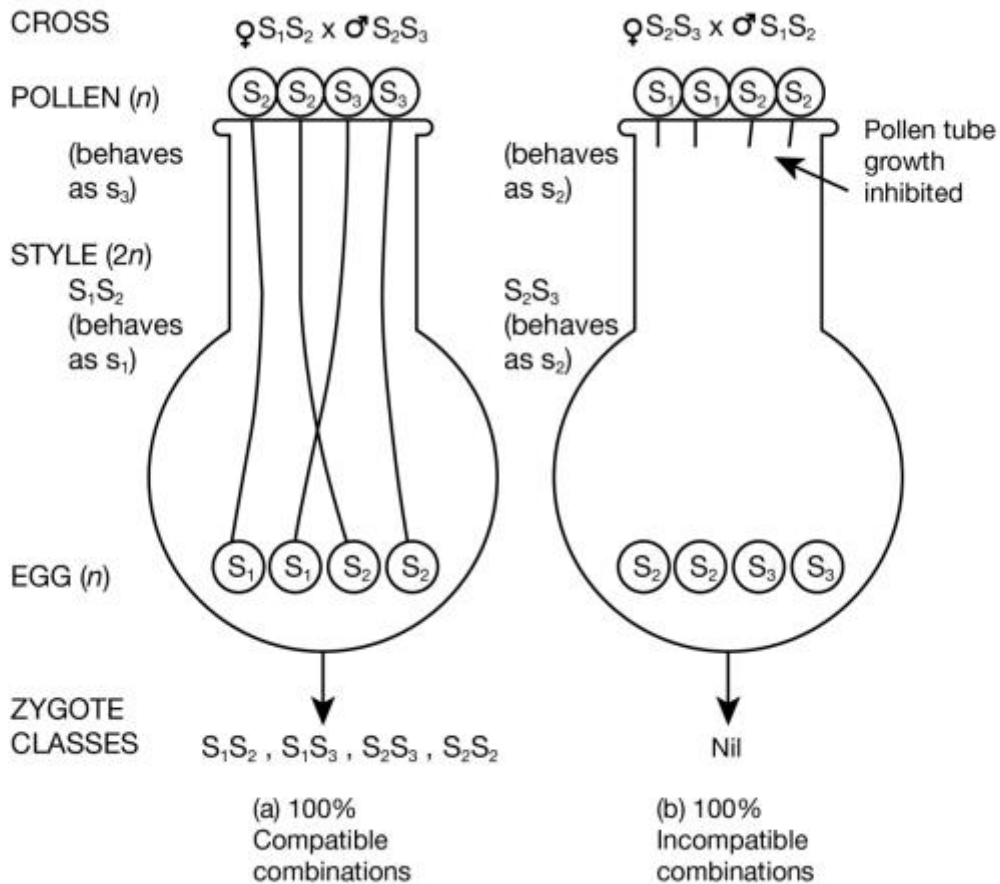


Figure 4.6 Differences in sporophytic compatibility between the crosses ($\text{♀}S_1S_2 \times \text{♂}S_2S_3$) and its reciprocal when the order of dominance of alleles in the style is $S_1 > S_2 > S_3$ but is reversed in the pollen, i.e. $S_3 > S_2 > S_1$.

Sterility

Sterility occurs in most, if not all, crop plants at a very low frequency. The two types of sterility of interest to the plant breeder are genetic male-sterility and cytoplasmic male-sterility. Male-sterility is required to devise cheap and efficient breeding methods for exploiting hybrid vigour for commercial production of F_1 -hybrid varieties, both cross-pollinated and self-pollinated. These forms of sterility enable the breeder to render the female parent of a cross fully sterile in unlimited numbers. It allows large amounts of F_1 seed to be produced cheaply. This is done by planting the male parent in close proximity to the female (= male-sterile) parent, allowing the male parent to pollinate the male-sterile parent. The F_1 hybrid seed is then harvested only from the male-sterile parent. Commercial production of hybrid (F_1) seed has only become practicable since the use of these male-sterility mechanisms. It would not be practical to do this if the breeder had to rely on the laborious method of hand emasculation to achieve hybrid seed in the amounts necessary for commercial production

Genetic male-sterility Genetic male-sterility occurs in low frequency in most crop plants and is often conferred by the action of a single recessive gene, denoted *ms*. In the homozygous recessive condition, *ms ms*, plants are completely male-sterile. Its use in breeding involves incorporating the male-sterile gene in the female parent of a

proposed cross, usually by backcrossing. One problem with the use of genetic male-sterility is that the genetic male-sterile condition has to be maintained by crossing the male-sterile line with the same line of normal fertility. Therefore, in using the male-sterile line in a breeding scheme the maintainer line will contain male-sterile and male-fertile individuals. A considerable amount of work has been carried out to find genes for vegetative characters which are very closely linked to the male-sterile locus so that before flowering, the homozygous dominants, $Ms Ms$, and heterozygotes, $Ms ms$, can be rogued out of the female parent rows leaving only $ms ms$ plants for pollination by the male parent (Figure 4.7).

One problem with the use of male-sterility in breeding, particularly with regard to self-pollinating species which have not been selected for cross-pollination (either by wind or insects), is the low level of cross-pollination able to be achieved on to the male-sterile line. This might be improved if it were possible to alter floral morphology towards a more exposed stigma or to choose environments where climatic conditions might favour higher levels of cross-pollination.

Cytoplasmic male-sterility This form of male-sterility is conferred by the cytoplasm and its expression is dependent on the action of a gene which in the homozygous recessive condition allows male-sterility to be expressed, but in the heterozygous, or homozygous dominant, condition the plant maintains normal fertility. This mechanism of sterility induction has been found in a large number of crop plants, both cross-pollinated and self-pollinated, and has been of significance in the large-scale production of hybrid seed for the commercial exploitation of heterosis in the F_1 plant. Cytoplasmic male-sterility was first used for the production of hybrid seed in onion. A single male-sterile plant was found in the variety Italian Red which, when hybridised with a number of plants of normal fertility, gave three types of breeding behaviour in the progeny of the crosses. One type of progeny gave all male-sterile plants, another male-fertiles:male-steriles in the ratio 1:1, and the third type all male-fertiles. These results were accounted for by the presence in the original male-sterile plant of a sterility-inducing cytoplasm, denoted S , whose influence was expressed by virtue of a double recessive gene for sterility, rr , on a chromosome of this plant. Its cytoplasm-gene designation was $S rr$. The plants of normal fertility which in hybridisation with the original male-sterile plant gave the three types of progeny – all male-steriles, male-sterile : male-fertile (1:1) and all male-fertiles – had cytoplasm-gene constitutions of $S rr$, $F Rr$ and $F RR$ respectively. The presence of the fertile cytoplasm F gave male-fertility regardless of the type of alleles at the fertility/sterility locus on the chromosome. Thus the possible cytoplasm-gene combinations for fertility or sterility are as follows:

$F RR$ – male-fertile	$S RR$ – male-fertile
$F Rr$ – male-fertile	$S Rr$ – male-fertile
$F rr$ – male-fertile	$S rr$ – male-sterile

In maize, the R type genes have the capacity to restore fertility and are called restorers. One common source of cytoplasmic male-sterility in maize is that derived from the variety Mexican June and is referred to as the Texas-type of male-sterility. Fertility can be restored to this type of sterility by two dominant genes, $Rf1$ and $Rf2$.

The gene *Rf2* is found in nearly all forms of maize, and two genes in combination will completely restore fertility. The mechanisms of cytoplasmic male-sterility and fertility restoration that occur in maize had been found in a number of other plants, including wheat, linseed, tobacco, sorghum and millet.

METHODS OF HYBRIDISING CROP PLANTS

Hybridisation is used in the second phase of a breeding program to bring together genes of specially chosen parents, often from diverse sources, to produce new genetic combinations. These combinations are likely to give much greater potential for genetic improvement than selection from the existing populations alone (especially in self-pollinating species). Hybridisation is, therefore, a key activity in plant breeding.

In predominantly self-pollinated plants, such as wheat, barley, canola and lupins, the technique of artificial hybridisation involves the following steps:

1. If flowers are borne in inflorescences, they are examined to find those in which the earliest flowers are 2 to 3 days prior to anthesis (= release of mature pollen and fertilisation).
2. Those parts of the inflorescence bearing flowers which are too small or too late in maturity for hybridising are removed by dissection.
3. The anthers of each flower are removed using forceps. This is called emasculation. If the flowers are very small this operation is carried out using necessary optical magnification. The emasculated flowers are enclosed in a glassine bag fastened so that foreign pollen cannot enter. If flowers are borne singly in axils of leaves they can be covered individually with cotton-wool wrapping. The emasculated flowers are left for 1 to 2 days, the time for the stigma to become receptive, at which time anthers from the male parent are chosen immediately prior to anthesis. This is judged when the anthers are deep yellow and have not dehisced. In this condition they will dehisce soon after removal from the flower. Upon dehiscence, the pollen is dusted on the stigma with the anther held in forceps.
4. After pollination, the inflorescence or the single flowers are again bagged, or wrapped in cotton wool.
5. The bags can be removed from the flowers about 10 to 12 days after pollination and the hybrid F_1 seed allowed to ripen.

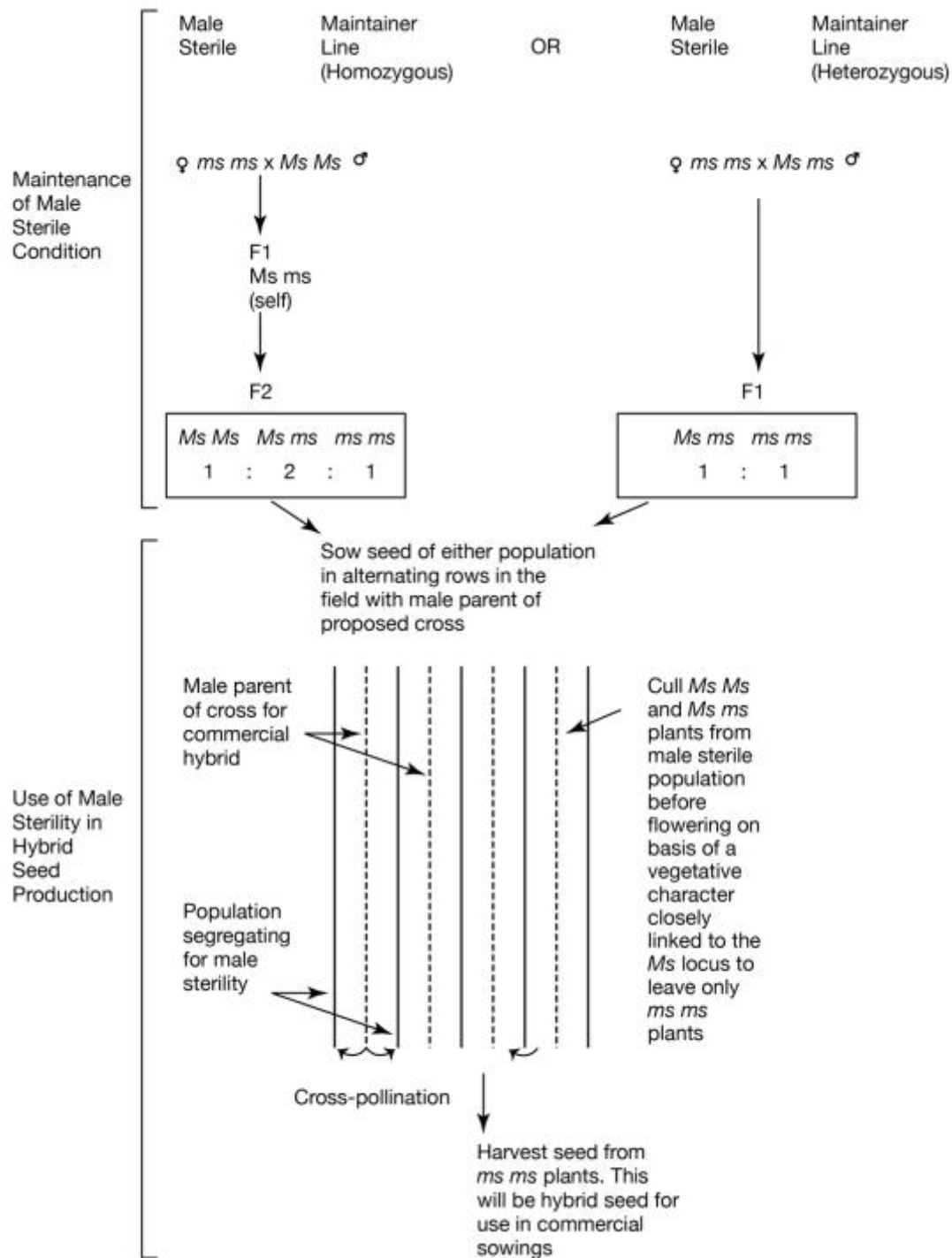


Figure 4.7 Outline of a scheme for maintaining genetic male sterility and using it in a breeding program to produce hybrid F_1 seed

For cross-pollinating species which are monoecious, and those hermaphroditic (i.e. having bisexual flowers) species which are strongly self-incompatible, emasculation is not necessary prior to hybridisation. The hybridisation is as follows:

1. Female flowers of the female parent of the cross are enclosed in glassine or paper bags well before the stigma(s) becomes receptive. This prevents

self-pollination or within-parent pollination for the monoecious and dioecious parents respectively, and also prevents cross-pollination with other adjacent plants.

2. When the stigma(s) becomes receptive, pollen is collected from the male flowers of the male parent of the cross. The pollen is collected when the male flowers have just reached anthesis; if the male inflorescence is many-flowered, the pollen can be collected by shaking the inflorescence into a paper bag.
3. Pollen is then placed, or dusted out of the bag, onto the stigma(s) of flowers of the female parent. The pollinated flowers are again bagged to prevent pollination from foreign pollen.

Detailed descriptions for hybridising a wide range of plant species are given in Fehr and Hadley (1980).

After the F_1 seed is harvested it is germinated and grown-up to produce as large an F_2 population as possible. This ensures maximum opportunity for recombination and segregation to produce new gene combinations. If a more complicated hybridisation is required, then the F_1 plants may be used directly as a parent in further crosses. In some instances, the segregating F_2 plants are selected and only the desirable individuals are used in backcrosses (see below) or other hybridisations.

Plant breeders use a standard, compressed method of representing the pedigree of a particular genotype (Purdey *et al.*, 1968). The details included are the parents, the crosses made, and the selections made to produce the line. The system facilitates the electronic storage of pedigree information and is often used with local (program-specific) modifications. A simple example of a pedigree describing the origin of a genotype is:

(Merrit-8/Jindalee//WL747*2)-5-0-0-0-63-0-0

This means that a single plant selection number eight from variety Merrit (in this case the species is narrow-leaf lupins) was used as a female in a cross with the variety Jindalee as the male. The F_1 plants were used as females in a cross with the un-named male line WL747. The resulting three-way F_1 (as female) was crossed again with WL747 as the male (i.e. back-crossed). The segregating F_2 population was selected as single plants – this genotype was derived from plant number five. The progeny were bulked in generations F_3 , F_4 and F_5 . In F_6 single plant number 63 was selected and its progeny bulked in F_7 and F_8 to produce the genotype in question.

SELECTION IN CROP IMPROVEMENT

From the beginnings of agriculture humans have been, in a sense, plant breeders, in that they have exerted a selection pressure on the crop for both improved and more assured productivity. Their selection has also been qualitative in that they have found certain variants of the crop species especially suitable for particular uses. This is evident in a crop like maize where types especially suited to boiling, cooking, popping and colour variants for ceremonial use have been selected and retained as discrete types. Another example of the influence of human selective preference in crop

domestication is the choice of one particular crop over others for main use. The pre-eminence of wheat as the main cereal, over barley, oats and cereal rye, which had somewhat similar ranges of adaptiveness and yield potential as wheat, is an example of human discernment in selection. It is likely that the choice of wheat was that of palate preference in that the wheat grain possessed a protein complex, called gluten, which was unique in physical properties such as extensibility and resilience and in consequence produced a bread much more pleasing to the palate than the other cereals.

The source of all new genetic variability in the wild is mutation. New mutant genes are subject to selection and, if advantageous, find their way via hybridisation (rare in self-pollinators, common in cross-pollinators) into the cultivated forms of the species. Inbreeding crops consist mainly of large numbers of genetically-fixed lines, whereas out-crossers are usually present as genetically diverse populations. F₁-hybrid varieties are a special case where both genetic homogeneity and heterozygosity are maximised.

There are two main methods of selection in both self-pollinated and cross-pollinated plants. One method, called *mass selection*, is similar in procedure in these two groups of plants; it has been of significance in crop plant improvement, both throughout the history of crop domestication and in recent plant breeding programs. The other method of selection for self-pollinated plants is *pure line selection* and for cross-pollinated plants *progeny selection* and *line breeding*. It involves selecting single plants from crop populations and evaluating their progenies for superior performance. From this selection, lines or varieties which are genetically improved for the character(s) being selected can be produced. Owing to the different population structures in self-pollinated and cross-pollinated crops, the implications and procedures in selection are somewhat different. Because the homozygous condition is achieved through selfing in self-pollinated crop plants, single plant selection can be practised from which homozygous pure line varieties can be produced. In cross-pollinated crops, the heterozygous nature of all the individual plants in the population means that single plant selection would lead to inbreeding and homozygosity and a consequent loss in vigour and fertility. Selection in these latter crops therefore has to be based on a population of plants where out-breeding and hence heterozygosity is maintained at a high level.

Mass Selection

Mass selection involves the selecting of a group of individuals from a population on the basis of their similar phenotype in an attempt to improve the performance of the population for that character or characters. A knowledge of the genetic basis and the extent of the population variability for the character indicates to the crop breeder the potential offered for improvement through selection. In the mass selection procedure there is no progeny test (in contrast to pure line selection, and progeny selection and line breeding) but the selected population is usually evaluated against the original unselected population to gauge the effectiveness of selection.

In self-pollinated crops, mass selection has two main uses. Firstly, it is an efficient method of bringing rapid improvement to populations which have a proportion of individuals that are obviously (i.e. visually) unfit, such as too early or late in maturing, disease susceptible or lodging susceptible. Mass selection is used in this way to 'purify' populations for a narrower range of character value(s), a practice which is believed to confer closer-to-optimum fitness for that character, or characters, in commercial production. It is also used in pure seed production in self-pollinated crop varieties where selection is based on discarding phenotypic variants that diverge from the standard type, or range, for the variety.

In cross-pollinated crops, mass selection has contributed substantially to their improvement, particularly for characters of high heritability such as disease resistance, date of maturity and protein or oil content of grain. There are numerous examples of its successful application. Mass selection is, however, not an efficient method for improving characters of low heritability such as yield (see below).

Pure Line Selection

The pure line selection method depends on the knowledge that continued selfing of a heterozygous individual or population of a self-pollinated plant species will result in an increasing proportion of the subsequent population becoming homozygous. Eventually all individuals will be homozygous. In crop breeding the parents of a cross usually differ by a large number of gene pairs and this number will influence the number of generations of self-fertilisation necessary for the hybrid population to reach homozygosity. At this point the population would be heterogeneous and composed of a large number of different homozygous individuals. The degree of homozygosity of the hybrid population depends on the number of independent gene pairs by which the parents differ and the number of generations of selfing since hybridisation.

The proportion of the population which is homozygous is given by the formula

$$\left(\frac{2^m - 1}{2^m} \right)^n$$

where m = the number of generations of self-fertilisation; and n = the number of independent gene pairs by which the parents differ. Thus, if the parents of a cross differ by 12 independent gene pairs, the proportion of homozygosity of the hybrid population after 6 generations of self-fertilisation would be approximately 83%. The pure line method is the basis of selection in breeding programs for improvement in self-pollinated crops, such as wheat and barley.

Progeny Selection and Line Breeding

Progeny selection and line breeding has been an important method of improving the performance of cross-pollinated crops over the last 70 years. It involves selecting plants on phenotypic appearance from open-pollinated populations, selfing them and evaluating their progenies, usually as small rows or plots of 10 to 50 plants, in the following year. The inferior progenies are eliminated and the selected progenies are

incorporated in a composite population which should be genetically superior to the original population for the character, or characters, being selected.

The procedure of incorporating the selected superior progenies in a composite population is referred to as line breeding. The risks of inbreeding can be minimised by firstly incorporating a large number of progenies, usually greater than 50, in the composite population and secondly ensuring that they are genetically dissimilar. An outline of the progeny selection and line breeding method is shown in Figure 4.8.

This method of selection has proved useful for improving the performance of cross-pollinated crops for qualitative characters and also quantitative characters such as yield in unadapted populations. However, in highly adapted populations it is not an efficient method for improving yield, due both to low heritability and complex genetic make-up of the yield character.

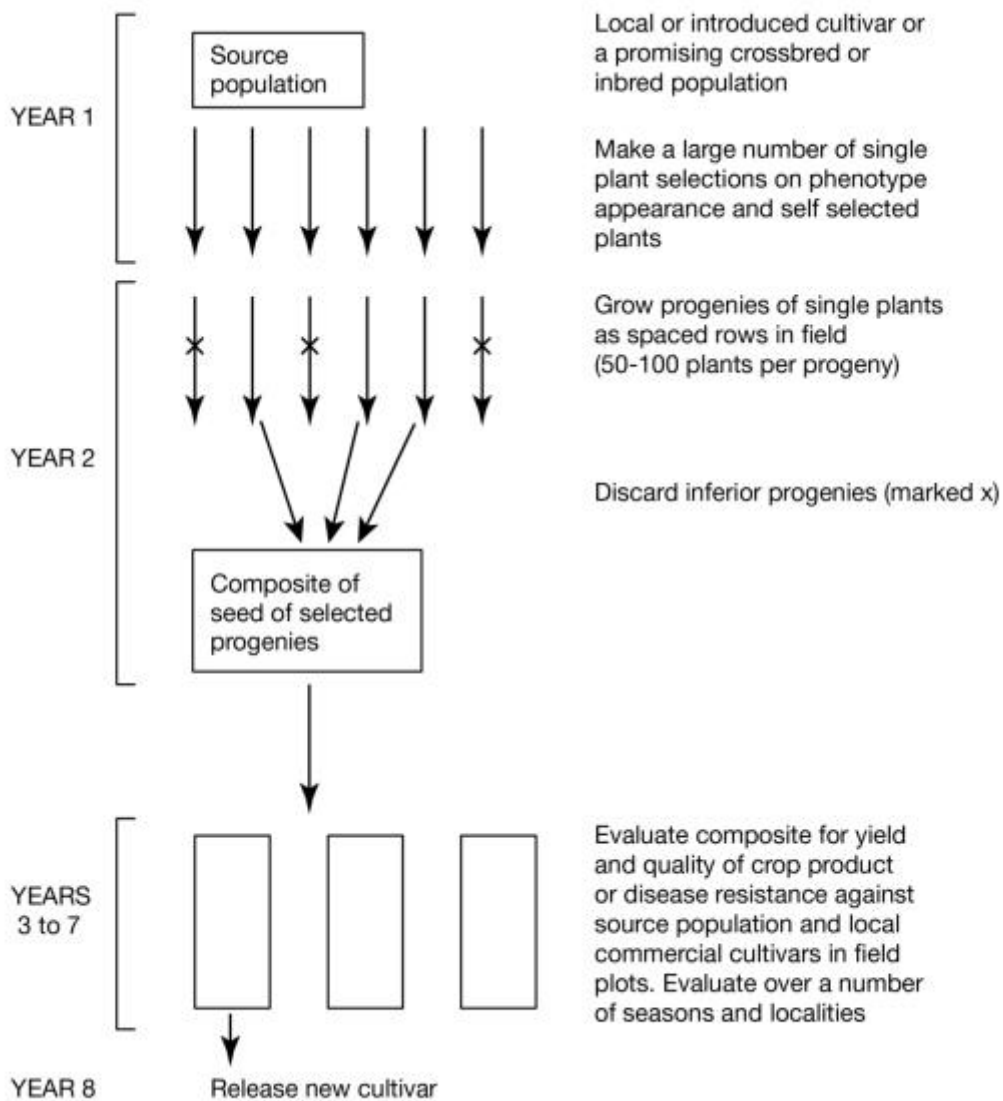


Figure 4.8 Progeny selection and line breeding procedure

Natural Selection

While most breeding programs in self-pollinated and cross-pollinated crops depend mainly on artificial selection for character improvement, there has been some interest in the possibility of using natural selection to achieve improvement in crop breeding.

Its use applies particularly to adaptive characters such as cold or drought tolerance and disease resistance or tolerance. Natural selection is allowed to act on large, segregating populations of self-pollinated crops, e.g. 'composite cross' populations of barley, or normal populations of cross-pollinating crops, when grown for a number of seasons in a particular environment. Under natural selection, significant shifts can be obtained in gene frequency towards greater adaptiveness, and hence increased or more reliable yield of the crop in that environment.

BREEDING AND SELECTION METHODS IN SELF-POLLINATED CROPS

Breeding and selection methods for self-pollinated crops are based on the knowledge that the genetic variability produced through hybridisation and recombination between carefully selected parents provides scope for obtaining more favourable recombinations of characters, and that it is possible to obtain homozygous lines containing these recombinants through selfing and selection. There is a number of methods for breeding and selecting self-pollinated plants. In the choice of a particular method the breeder considers the genetic control of the character, i.e. whether simple or complex in inheritance, whether it is of high or low heritability, the degree of linkage with undesirable characters (if known), and the time, labour and space available in the breeding program.

The main methods used for breeding self-pollinated crops, and described here, are the pedigree, multiple cross, bulk, backcross, and single seed descent methods.

Pedigree Method

The pedigree method has found wide acceptance amongst breeders of self-pollinated crops (Figure 4.9). It typically involves the hybridisation of two parents, one usually a commercial variety and the other chosen because of a particular superior attribute. The breeder aims to incorporate this attribute in a genetic combination that is at least equal to the commercial variety in all characters and possessing the attribute of the donor parent. The pedigree method involves selecting single plants from segregating populations of a cross and evaluating the performance of their progeny with repeated selection within the better progeny for single plants followed again by further progeny evaluation. Single plant selection can be commenced as early as the F_2 population or it can be delayed until the F_3 or F_4 generation from the bulk segregation population. Single plant selection followed by progeny evaluation is followed until the F_6 or F_7 generation, by which time most of the lines are homozygous and can be judged visually for phenotypic uniformity. At the F_7 the homozygous selected lines can be placed in replicated, multi-location yield trials for further selection on the basis of yield performance. Some breeders employ F_2 single plant selection and then select single

plants again at F_5 or F_6 . This approach produces extremely uniform varieties but takes time especially in species with low seed-multiplication rates (e.g. pulses).

The pedigree method is useful for handling a large number of recombinants with comparative ease. The crop breeder usually selects in early generations, on a visual basis, for qualitative characteristics such as height, standing ability, morphology of the inflorescence, disease resistance and seed characters such as shape, colour and size. Because most of these characters are of reasonably high heritability and under simple genetic control this selection is very efficient in improving the overall agronomic suitability of the resultant population. Selection in later generations can be commenced for quantitative characters such as yield and quality. Because there are fewer selected lines and greater amounts of seed of the selections, small replicated trials are suitable for the reliable evaluation of such characters. Many studies have been conducted on the efficiency of selecting for yield on a single plant, or row, basis in early segregating generations but it is not considered to be a reliable method.

The theory behind the use of the pedigree method of selection bears upon its practicability in enabling the breeder to handle a very large number of recombinants in early segregating generations. When the breeder is using parents which differ by a large number of gene pairs, even when the character being selected is under simple genetic control, the possible number of recombinant types for the overall parental differences is very large. If the parents differ by n allelic pairs of genes with full dominance the kinds of possible phenotypes in the F_2 generation are 2^n . Thus, for a difference between parents of 20 allelic pairs of genes the number of possible phenotypes in the F_2 is 2^{20} (i.e. 1,048,576). Although the breeder can never expect to grow populations that reflect these huge numbers, experience and computer-simulation study have shown that the pedigree method offers a reasonably good chance of isolating a near-best genotype with about 90% of the desirable genes available from the two parents of a cross.

Multiple Cross

This method of breeding is similar to the pedigree method except that it involves the hybridisation of more than two parents, sometimes from 4 to 8. It has been used in attempts to combine the attributes for a particular quantitative character, such as yield or grain quality, from a number of adapted parents of divergent genetic origins. An outline of the method is shown in Figure 4.10. One severe limitation in the use of the method is the large number of crosses which would have to be performed especially in the eight parent cross (i.e. in hybridising the two double F_1 s) in order to have a good chance of adequately sampling the possible range of recombinants. In other words, the F_1 parents will be segregating to produce F_2 gametes and this gamete population must be adequately sampled in the crossing to ensure that as many gene combinations as possible are represented. The subsequent selection procedure could be that shown for either the pedigree or the bulk selection method.

Bulk Method

The bulk method of breeding involves hybridising two chosen parents and growing the F_2 and subsequent generations until the F_6 generation in bulk populations as field plots in successive years (Figure 4.11). During this time selection is usually not practised but in the F_5 or F_6 a large number of single plant selections are taken and their progeny sown as individual rows in the following season. Progenies can be culled on agronomic performance, including height, standing ability, threshing characteristics and such characters as flowering time and grain protein content. The progeny rows which are selected and which appear to be homozygous, as judged on the phenotypic uniformity of the row, can be evaluated for yield in small-plot replicated trials in the following year.

The bulk method of breeding relieves the breeder of the large amount of work in sowing, selecting and harvesting single plant selections and progeny rows from F_2 to F_6 as is the case with the pedigree method. It has the advantage of enabling the breeder to handle a large number of crosses because of the relative ease in bulk sowing and harvesting the segregating populations. The comparative evaluation of bulk yields of the segregating populations of different crosses has been used to indicate those crosses wherein selection for yield might provide the highest yielding segregates.

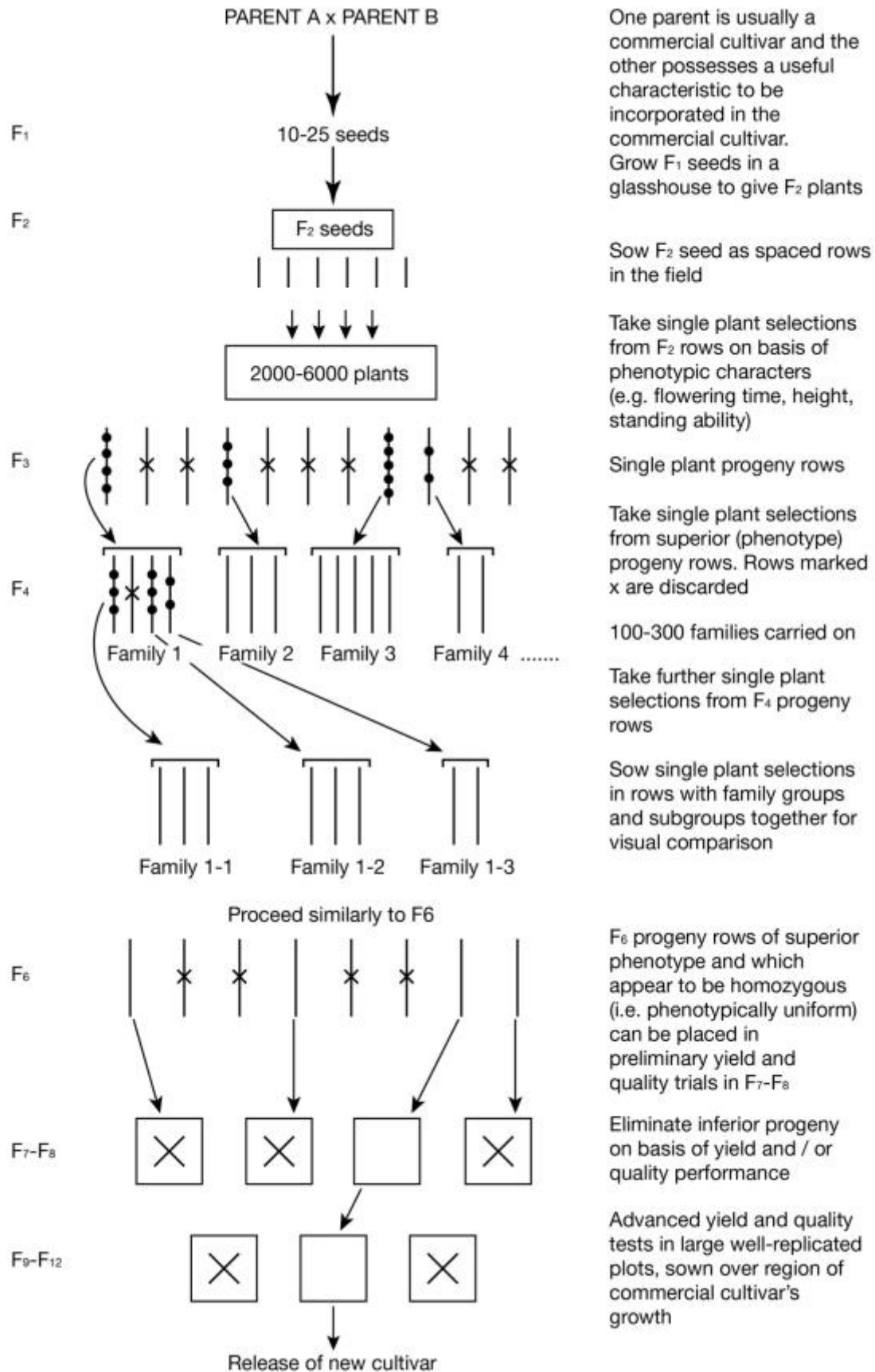


Figure 4.9 Pedigree method of breeding

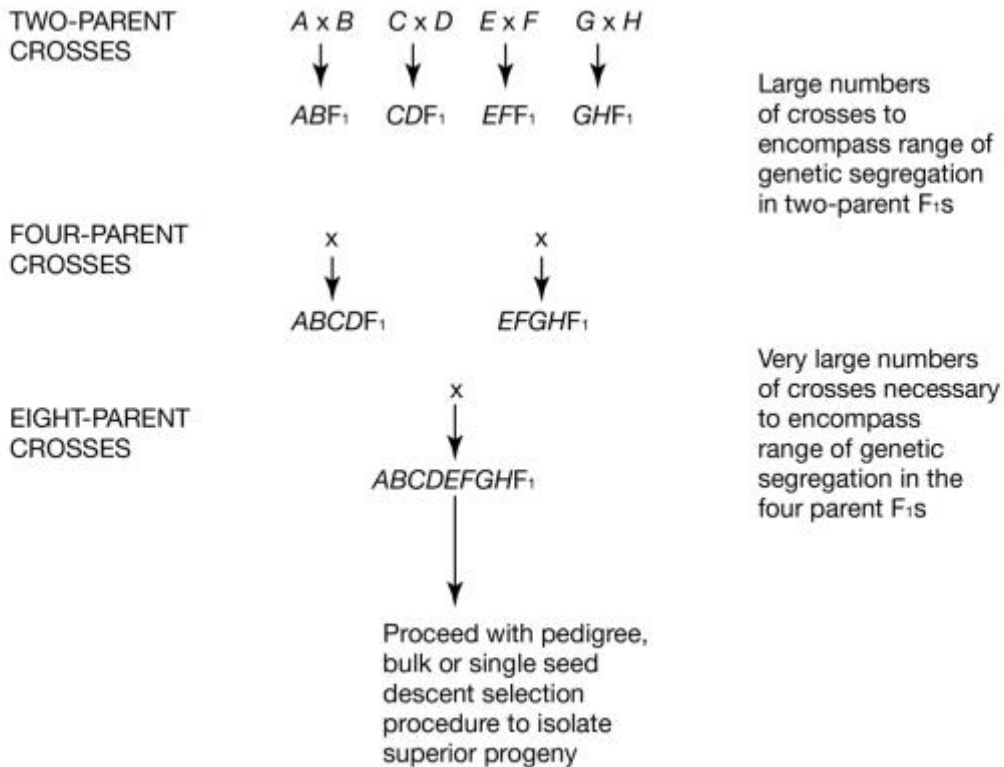


Figure 4.10 Multiple cross breeding procedure

Backcross Method

The backcross method of plant breeding is used for incorporating a gene, or genes, from a donor parent (called the non-recurrent parent) into an adapted variety (the recurrent parent). This is achieved by crossing the variety with the donor parent and then crossing the variety again with the F₁ of the first hybridisation. The resulting seed is called the first backcross F₁ seed, or BC₁F₁.

Segregates of the first backcross generation carrying the desired character of the donor parent are hybridised with the recurrent parent. This is called the second backcross. This procedure of selection and hybridisation with the recurrent parent can be continued until the sixth backcross at which stage the backcross line will be genetically almost identical with the recurrent parent but bearing the desired character of the donor parent (Table 4.4). The selection procedure during backcrossing will differ according to whether the character being transferred is controlled by one or more dominant or recessive genes. An outline of the two different procedures is shown in Figure 4.12.

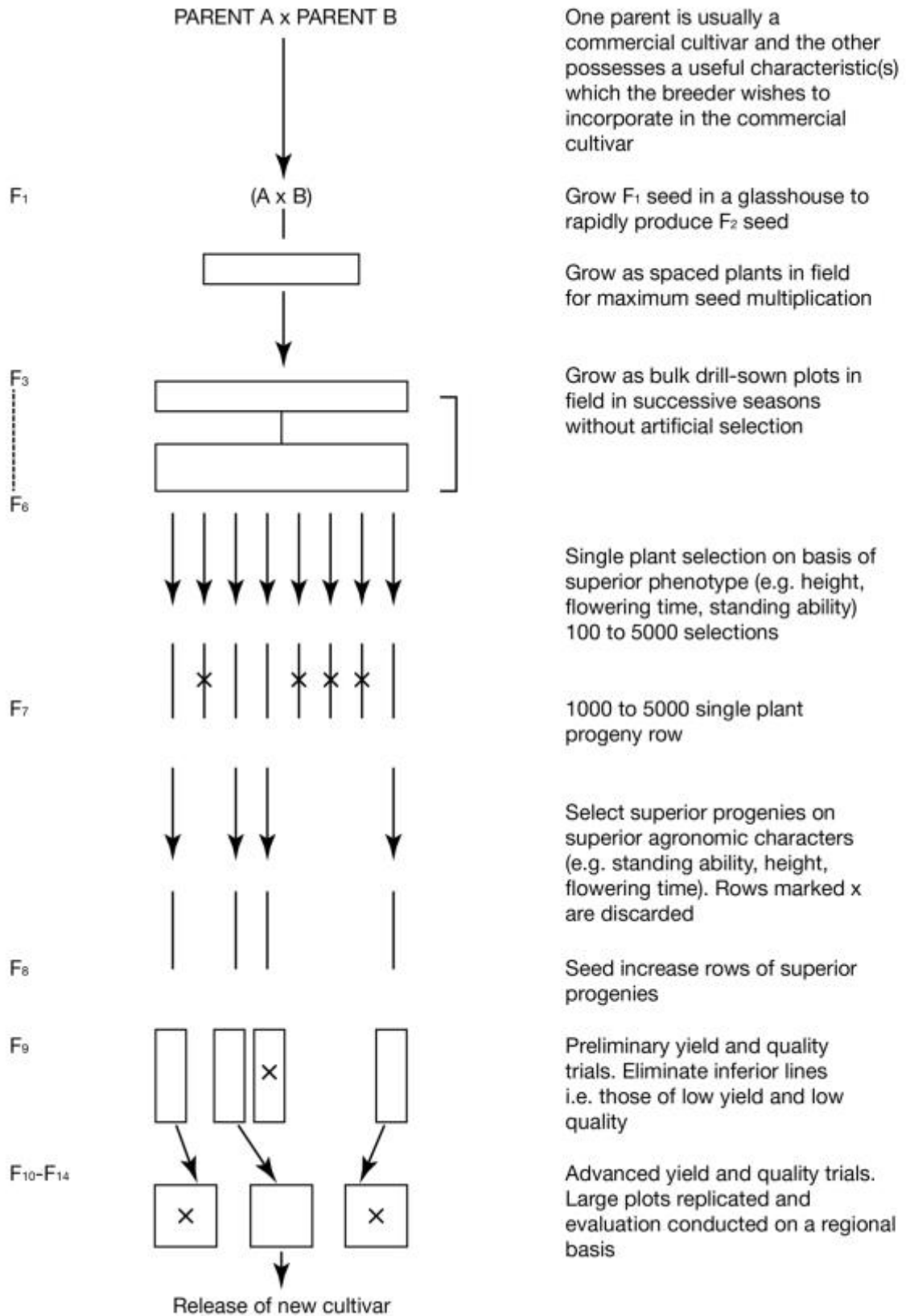


Figure 4.11 Bulk population breeding method

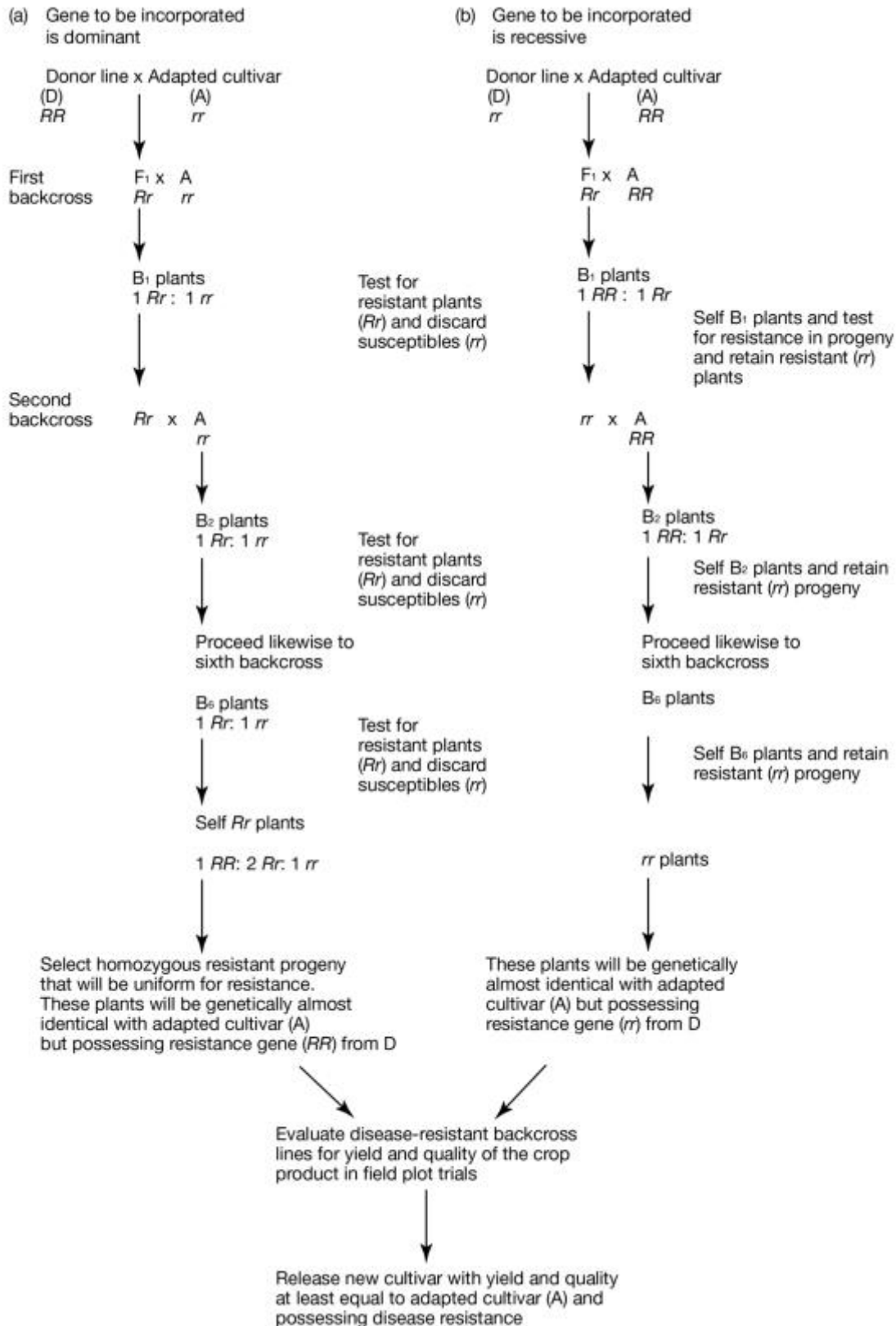


Figure 4.12 Backcross breeding method according to whether the gene to be incorporated is (a) dominant or (b) recessive

Table 4.4 Relative constitution of the backcross population for the gene content of the recurrent and non-recurrent parents with successive backcrosses

Cross	Donor line (D) x adapted cultivar (A) (non-recurrent x recurrent parent)	Relative gene content (%) of backcross generation	
		D	: A
First cross – F ₁	(D x A)	50	: 50
First backcross – B ₁	(D x A) x A	25	: 75
Second backcross – B ₂	(D x A ²) x A	12.5	: 87.5
Third backcross – B ₃	(D x A ³) x A	6.25	: 93.75
Fourth backcross – B ₄	(D x A ⁴) x A	3.125	: 96.875

The backcross method of breeding is more efficient than the pedigree method when there is close linkage between the desired gene and undesirable genes in the donor parent. Repeated backcrossing with the recurrent parent increases the chance of breaking such linkages. The effectiveness of backcrossing and selfing in breaking linkage, compared with continued selfing as in the pedigree method, depends on the degree of linkage and on the number of backcrosses performed. The probability through backcrossing of eliminating an undesirable gene which is linked to the desired gene in the donor parent is expressed in the formula

$$1 - (1 - P)^{n+1}$$

where P is the recombination fraction between the desirable and the undesirable gene and n is the number of backcrosses. The effect of varying degrees of linkage (recombination fractions) on the probability of eliminating an undesirable gene linked to a desired gene, after five backcrosses, is shown in Table 4.5. It indicates that backcrossing is more efficient in breaking linkage than selfing and its efficiency over selfing is greater when there is very close linkage between the desired and undesired genes.

Table 4.5 Comparative effectiveness of backcrossing versus selfing in eliminating an undesirable gene linked to a desirable gene

Recombination fraction	Probability that undesirable gene will be eliminated	
	With five backcrosses	With selfing
0.50	0.98	0.50
0.20	0.74	0.20
0.10	0.47	0.10
0.02	0.11	0.02
0.01	0.06	0.01
0.001	0.006	0.001

In reality, plant breeders have often used only two or three backcrosses, after which they select for both the retention of the specific character from the donor parent plus the possibility of increased yield over the recurrent parent. It is possible at this stage of backcrossing, when the populations are still reasonably heterozygous, that transgressive segregates for a character like yield may be selected.

Single Seed Descent

Single seed descent (SSD) is used in the breeding of self-pollinated plants by advancing lines from generation to generation through the use of a single seed from the progeny of each plant (rather than single plant and progeny row selection as in the pedigree method). An outline of the single-seed descent procedure is shown in Figure 4.13.

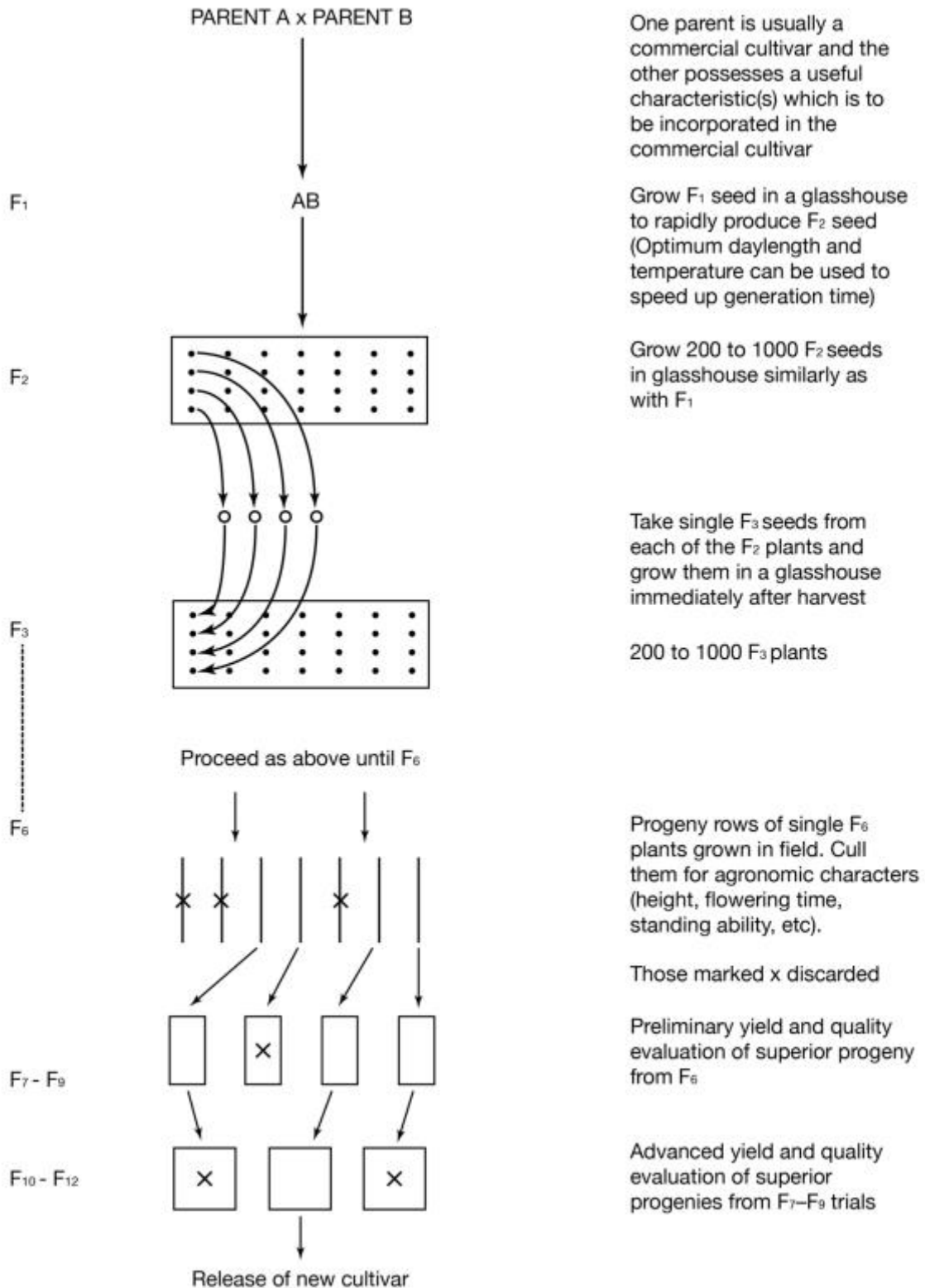


Figure 4.13 Single-seed descent breeding procedure

In most cases where SSD is employed the populations are grown as rapidly as possible in artificial conditions (glasshouse or growth room) so as to hurry through at least two generations per year and achieve near-homozygosity as quickly as possible. This is expensive and since the populations are still quite large it restricts the number of crosses that can be handled this way. Selection is rarely practised during this rush to homozygosity except for highly-heritable characters that can be cheaply and easily scored on single, crowded plants without risk of loss. Once homozygosity is approached, say F_5 , the lines are bulked-up and serious selection can commence. As soon as seed supply permits, field trials for quantitative characters can begin. While there is a time saving to be made with SSD, undesirable genotypes will persist in the breeding program for a greater number of generations before being eliminated.

BREEDING AND SELECTION METHODS IN CROSS-POLLINATED CROPS

Earlier selection methods in cross-pollinated crops (i.e. mass selection and progeny selection and line breeding) have certain limitations, particularly when used for improving highly-adapted varieties for characters of low heritability such as yield. Mass selection is of limited use for such characters, firstly because of the failure of phenotypic selection to isolate superior genotypes for the desired character and secondly because pollination is uncontrolled in that superior genotypes hybridise with inferior genotypes in the population being selected. Progeny selection and line breeding is a more efficient method in that it embodies progeny evaluation of selections and, particularly if done in replication, provides substantiation for the retention of superior genotypes.

However, these methods of selection have been found to be ineffective in improving yield in highly adapted genotypes. This is mainly due to the low frequency of favourable genes for yield in cross-pollinated populations, and the ineffectiveness of these methods in retaining genetic variability and allowing recombination to produce more favourable recombinants for yield within the population under selection. Recurrent selection (in its various forms) is a method to increase the frequency of favourable genes for yield (or any other quantitative character) by allowing crossing and recombination to take place only between the best performing selections. In cross-pollinating species, recurrent selection must be used while maintaining genetic variability in the population being selected to avoid inbreeding depression. Recurrent selection broadly involves the following steps:

1. individual plants are selected from an open-pollinated source population and selfed. These plants are evaluated at the same time for the character, or characters, being selected;
2. plants of inferior performance (assessed visually or on the behaviour of selfed progeny or test-cross progeny) are discarded;
3. selfed plants in Step 1 with superior progenies are propagated from the retained portion of selfed seed;
4. superior progenies are placed in a crossing block to obtain as many intercrosses between progenies as possible;
5. the intercross population is sown as a basis for further cycles of selection and intercrossing as in Steps 1 to 4.

There are three main methods of recurrent selection – simple recurrent selection, recurrent selection for combining ability, and reciprocal recurrent selection; they are each discussed briefly below.

Simple Recurrent Selection

This method of selection involves visually selecting single plants from an open-pollinated source population and selfing them. The progenies of the selections are sown in a crossing block in the following year and allowed to freely intercross. The intercross population is sown in the following year and single plant selection and selfing carried out again. The progenies of the selfed plants are sown again in the following year in an intercross block. The second cycle of selection and sowing of progenies is called the first recurrent selection cycle. The cycle can be continued until there appears to be no further significant advance being obtained for the character or characters under selection. Because the method does not involve progeny evaluation it is suitable only for characters of high heritability. An outline of the simple recurrent selection procedure is shown in Figure 4.14.

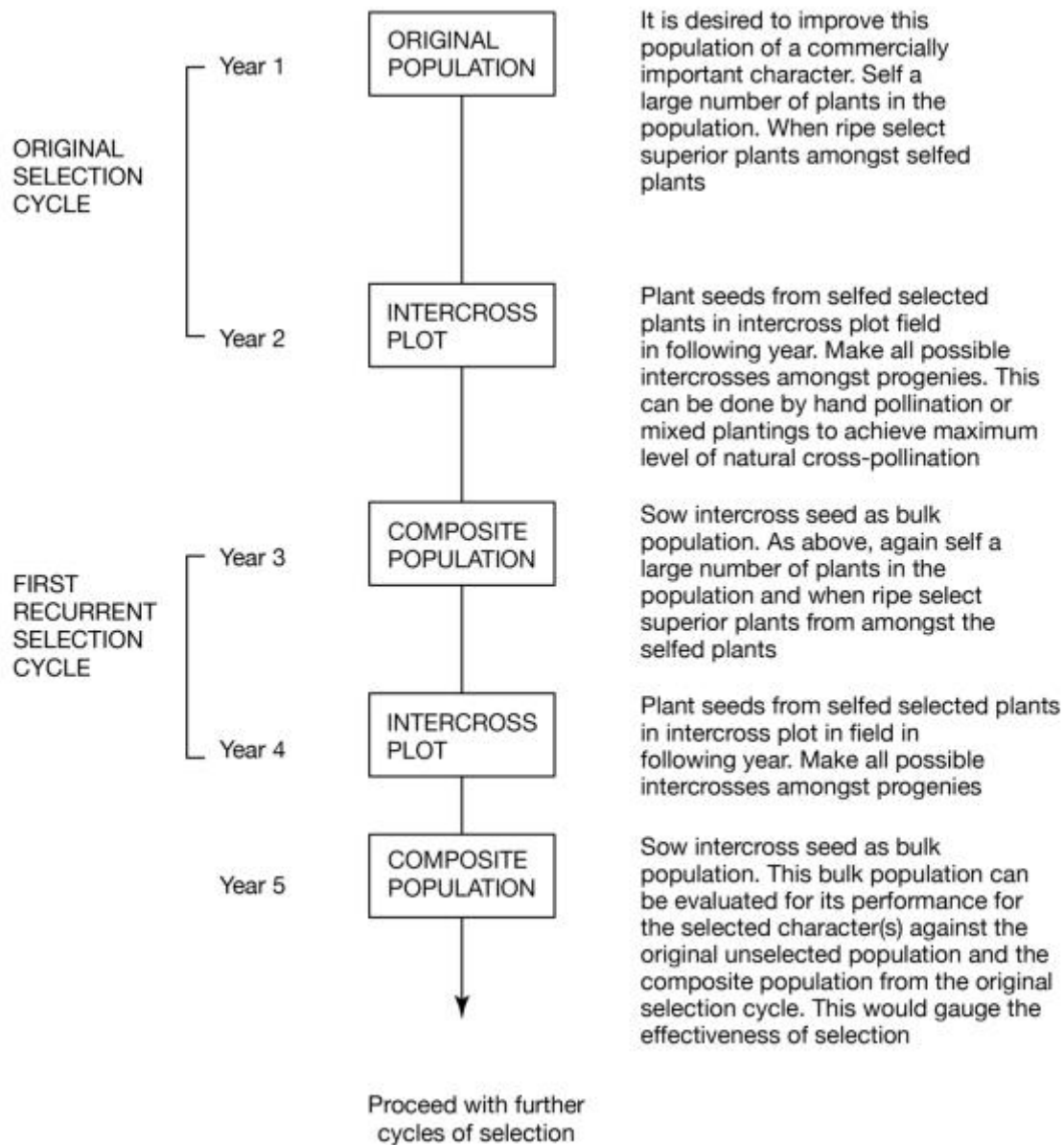


Figure 4.14 Simple recurrent selection

Recurrent Selection for Combining Ability

This method of recurrent selection involves selecting single plants (called S_0 plants) from a source population, selfing them and, at the same time, crossing them to a tester. The tester is either a heterozygous population or a homozygous line, and the progeny of the test crosses to it are evaluated comparatively for the character being selected. Crosses that result in high progeny mean performance is said to have good combining ability, either for one cross (specific combining ability) or generally across all crosses involving that parent (specific combining ability). Selfed seed from S_0 plants which are good combiners is then sown in a crossing block in the field and the progenies are either intercrossed by hand or allowed to intercross naturally. The harvested seed is then sown, and a further cycle of selection and test-crossing can be carried out. Recurrent selection for combining ability has been shown to be a useful method for improving yield and adaptability of a number of cross-pollinated crops.

Reciprocal Recurrent Selection

This procedure involves the use of two genetically unrelated open-pollinated populations, A and B. Random single-plant selection (S_0 plants), and their selfing, is carried out in population A and at the same time they are crossed with a random sample of plants from population B (Figure 4.15). The same procedure is also carried out in population B. The progenies of these reciprocal crosses are evaluated in replicated trials for the character or characters being selected. Selfed seeds of S_0 plants of the A and B populations that gives superior progenies in these crosses are then sown as separate A and B populations and allowed to intercross within each population.

The bulk progenies of the intercrosses are then sown as separate A and B populations, and a further cycle of selection is carried out. It may be decided after the second cycle either to proceed to the third cycle or to use the selected populations to produce commercial hybrids. This is achieved by making crosses between the selected A and B populations.

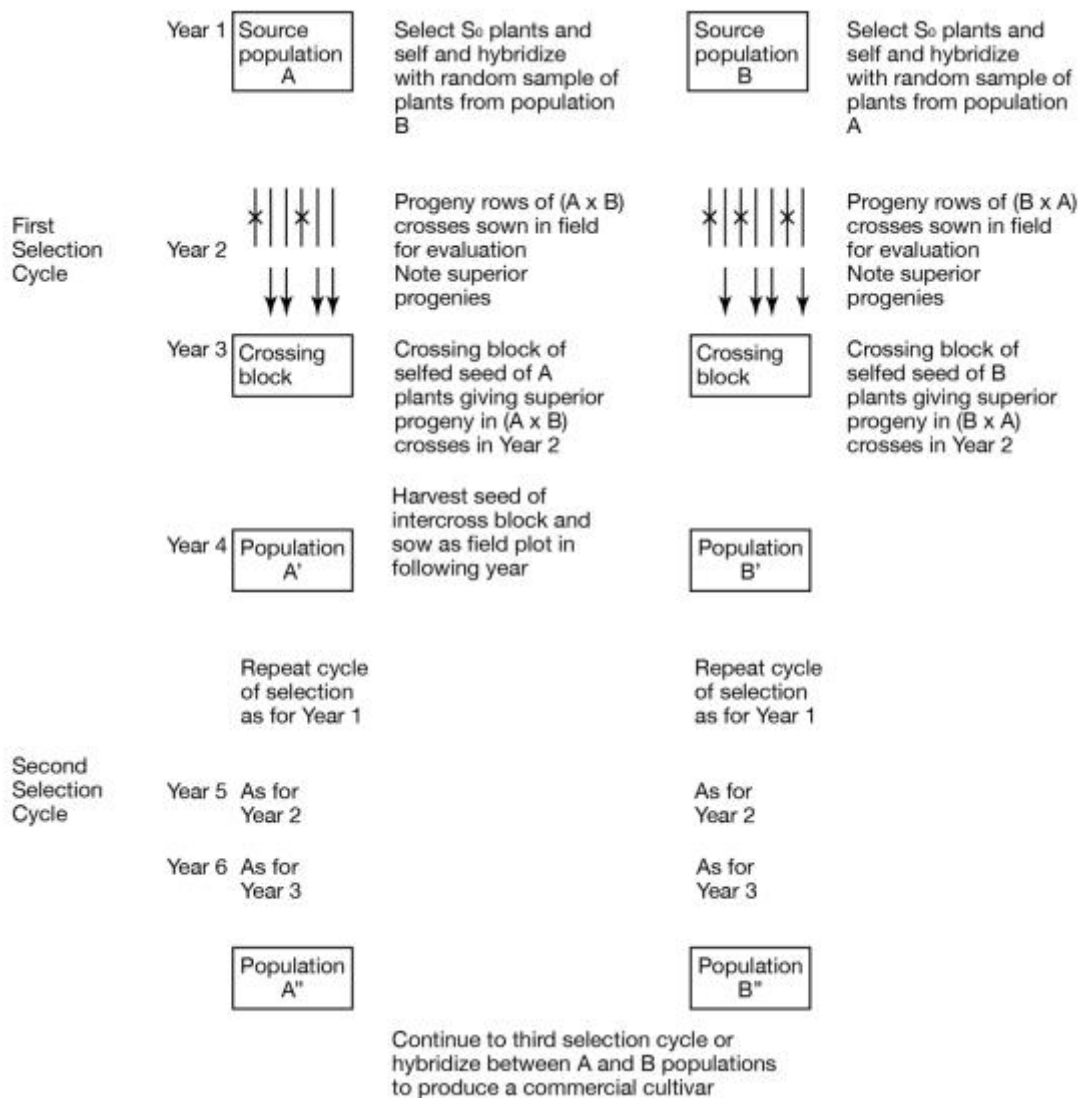


Figure 4.15 A procedure for reciprocal recurrent selection

Synthetics

Synthetic varieties have been produced in a number of cross-pollinated crops as a means of exploiting the heterosis of combinations amongst a set of specially chosen parents. An important condition for constituting a synthetic variety is that the parents must exhibit good combining ability for yield in all pairwise combinations.

The performance of advanced generations of a synthetic variety depends on three important factors: the number of parents entering into the synthetic; the mean performance of the parents for the character; and the mean performance of all combinations arising from hybridisation amongst the parental lines. These requirements can be expressed in the formula

$$\text{Syn2} = \text{Syn1} - ((\text{Syn1} - \text{Syn0}) / n)$$

where Syn2 is the estimated performance of the second generation (or F₂) of the synthetic after its constitution, Syn1 is the mean performance of all the hybrids (or F₁s) of the parents, Syn0 is the mean performance of all the parents, and n is the number of parents. Syn1 – Syn0 represents the extra performance of the first generation population over its parents. For example, a two-parent combination will have a second generation (Syn2) performance equal to

$$\text{Syn1} - ((\text{Syn1} - \text{Syn0}) / 2)$$

i.e. it will have lost half of the excess vigour of the Syn1 over the parents. With three, four, and five parents this decline in vigour will be 1/3, 1/4, and 1/5 respectively that of the Syn1, over the mean parental value. If the number of parents in the synthetic is made large, the loss in vigour of the Syn2 over the Syn1 should be very small. However, there is a limit to the number of parental lines which can be chosen to give high combining ability for yield. The optimum parent number has been shown to be approximately five to six for maize. Beyond this number the average combining ability declines.

Synthetic varieties are important in the commercial production of a number of cross-pollinated forage species like lucerne, and a number of pasture grasses such as perennial ryegrass, timothy grass and orchard grass. Combining ability tests must be made amongst potential parents to choose those that will genetically constitute the synthetic. Combining ability measurements are usually made on hybrids between the parents as a result of natural pollination. An important alternative method of assessing combining ability is the polycross test which is an evaluation of progeny of crosses obtained from random artificial hybridisation amongst the parental lines when planted together in the field. This latter approach ensures that all parental combinations are tested.

Once synthetic varieties of maize are established they can be maintained by mass selection and may even be further improved by selection.

Hybrid Varieties

A hybrid variety is an F_1 population grown commercially to exploit heterosis, usually for yielding ability. The double cross F_1 (i.e. four-parent hybrid) in maize with its increased yield over open-pollinated varieties has been one of the most significant plant breeding achievements.

The concept of hybrid varieties was first developed in maize – inbreds were produced and crossed in pairs. Those that gave superior F_1 performance were chosen for commercial production of hybrid seed. The advantages seen in the use of inbreds over open-pollinated varieties were that they were homozygous and were predictable in performance and they enabled the hybrid to be accurately reconstituted year after year. However, the single cross method of hybrid production did not succeed because the hybrids did not significantly surpass the yield of open-pollinated varieties. The seed was costly to produce because of the low yield of the inbred female parents and the low vigour and viability of the hybrid seed.

Later came the use of the double cross in maize, which is the hybrid between two F_1 s of four parents, i.e.

$$(A \times B) F_1 \times (C \times D) F_1$$

where A, B, C and D are four inbred parents. This method of hybrid production was more successful than the single cross hybrid. The single cross parents of the double cross were much more vigorous and higher yielding than the inbred parents of the single cross, and the hybrid seed was more vigorous and viable than the single cross seed.

The procedures used in producing hybrid maize are as follows:

1. select desirable plants from open-pollinated populations;
2. self the selections for several generations until homozygous to produce inbred lines;
3. hybridise chosen inbreds to produce single-cross F_1 s;
4. select those single crosses exhibiting the highest combining ability for the character(s) to be improved for use in the double-cross hybrids;
5. produce double-cross hybrids from the best-performing single crosses.

In the production of single-cross and double-cross seed, cytoplasmic male-sterility is used to minimise the labour otherwise necessary in detasseling (emasculating) female parents to produce the single and double crosses. Fertility-restoring genes are also used to improve the efficiency of producing the double-cross hybrid. An outline of two methods of producing double-cross hybrid maize using cytoplasmic male-sterility and fertility-restoring genes is shown in Figure 4.16. Four selected inbreds are hybridised through cross-pollination in pairs by growing the two pairs of parents together in the field and one pair in isolation from the other. A parent of one of the pairs, if it possesses cytoplasmic male-sterility, will transmit this to the single cross to make it male-sterile. One or both parents of the other single cross contain fertility-restoring genes and each is male-fertile so that their hybrid will also be male-fertile. The two single-cross hybrids

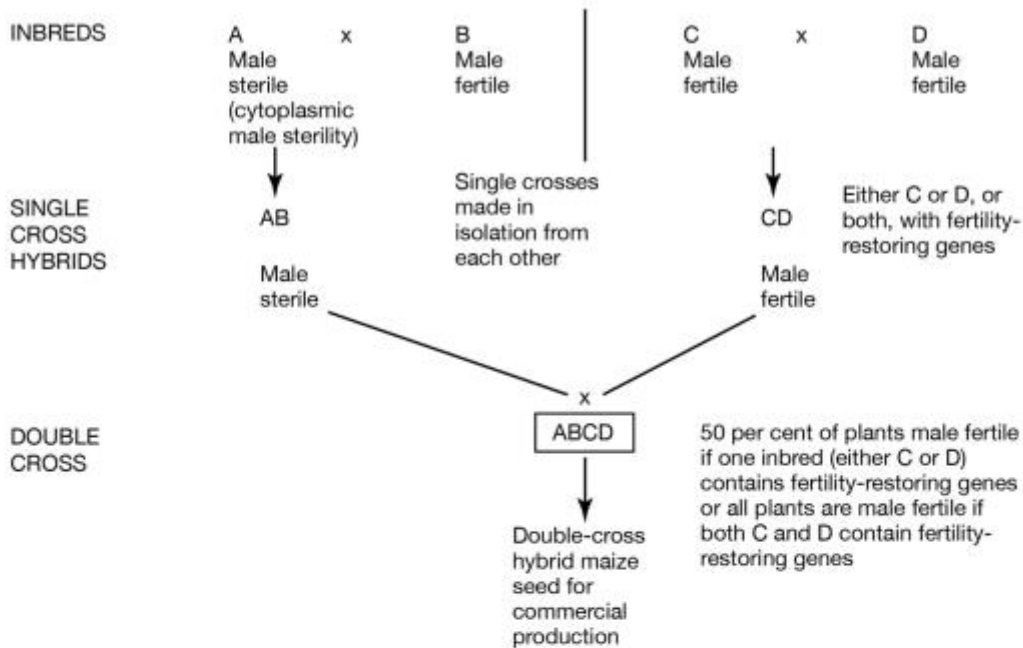
are then planted together in the field and cross-pollination takes place between the male-sterile parent and the male-fertile plant. The seed which develops on the male-sterile parent will be double-cross hybrid seed. The presence of fertility-restoring genes in the double-cross hybrid from one of the single-cross parents restores male-fertility in the double-cross population to 50% or 100% depending on whether one or both of the original parents of the single cross possesses restoring genes. The double-cross hybrid seed is grown commercially as hybrid maize.

Hybrid varieties are preferred by commercial breeders (as distinct from government-funded or public-good breeders) because heterosis breaks down in the F_2 and later generations due to segregation. Farmers must therefore buy new F_1 planting seed from the breeder (or the licensed seed producer) each season. In Australia, hybrid varieties have been a great success in maize, sunflowers, sorghum, and many vegetable crops.

PLANT BREEDING FOR SPECIFIC CHARACTERS

Breeding programs for improving particular characters in crop plants require a detailed knowledge, not only of the genetic control of the character but also of the type and magnitude of environmental influences on its expression. Environmental influences include climatic factors such as moisture availability and temperature, and edaphic factors such as nutrient availability and soil pH which act to influence the growth, productivity and quality of the crop. Plant breeding activities are broadly concerned with improving the yield and quality of the crop product and improving or maintaining the resistance of the crop to diseases (bacterial, fungal or viral) and insect pests.

(i) One inbred male sterile and either one or two inbreds with dominant fertility-restoring genes



(ii) One inbred male sterile and one inbred with dominant fertility-restoring genes

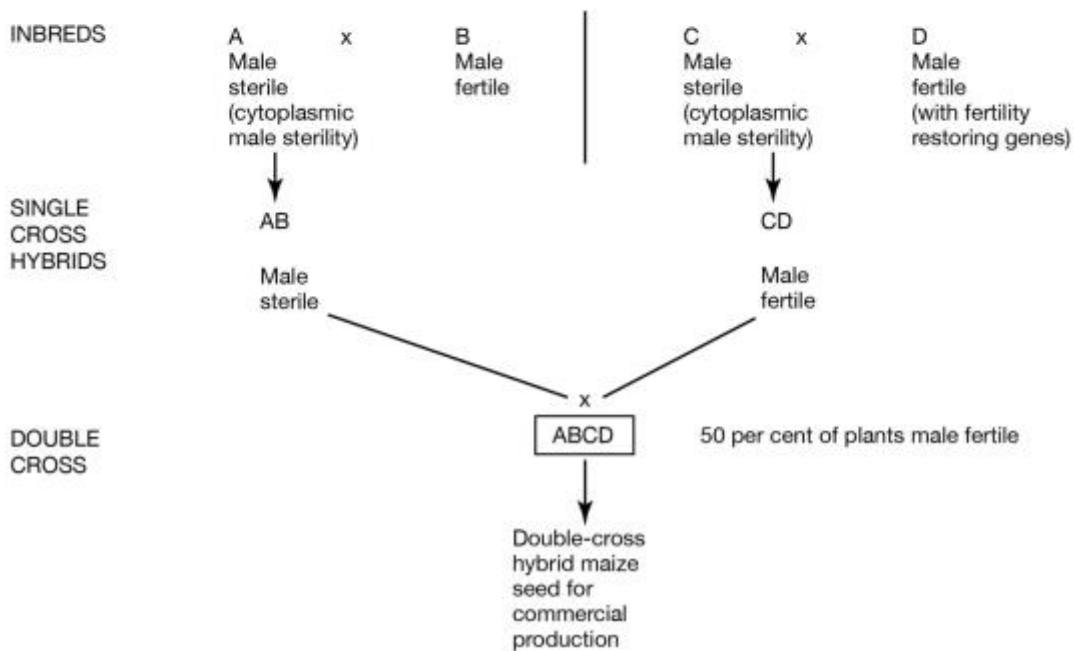


Figure 4.16 Two methods of producing double-cross hybrid maize seeds using cytoplasmic male sterility and fertility-restoring genes

Yield Breeding

Yield is the endpoint of the interaction of a large number of physiological and biochemical processes in the plant. Therefore its genetic control is complex and its heritability is usually low. Three main approaches may be discerned in breeding for increased yield in crop plants. First, yield can be regarded as a quantitative character,

in which case use is made of quantitative genetics in both the choice of potentially useful parents and in selection procedures. Second, certain approaches attempt to resolve yield into either its visible components (e.g. for wheat, grain size or weight, grain number per spikelet, number of spikelets per head and number of fertile tillers or shoots per plant), or physiological or biochemical processes, which may be under simpler genetic control than total yield. The third approach aims at increasing yield through selecting for increased adaptability, either generally over a range of seasons and localities, or specifically (e.g. improved cold or drought resistance, or greater tolerance to edaphic factors such as soil salinity or to extreme pH).

Several methods for the genetic analysis of yield have been devised for predicting the potential contribution to increased yield of parental lines in hybrid combination. An important method is the diallel analysis which involves the hybridisation of a set of n parental lines in all possible pair-wise combinations to give $1/2n(n-1)$ single crosses or $n(n-1)$ reciprocal crosses. The diallel analysis uses the character expression of F_1 and F_2 and later generations of all the crosses, plus that of the parental lines, to interpret the genetic control of yield and to indicate those crosses likely to give the highest yielding segregates.

Selection and testing of yield in replicated, multi-location trials leads to modern statistical analysis methods which partition the influences of environment into year (y), location (l) and $y \times l$ effects. These studies lead to estimates of heritability of yield and the selection intensity necessary to provide a reasonable chance of isolating high-yielding segregates from mixed populations. It also leads to the adoption of optimal trialing systems to maximise the use of available resources.

The component approach in breeding for increased yield is an area of great interest. Some of the visible components of yield (e.g. grain size and spikelet number in wheat) have been shown to have higher heritability than total yield and hence provide more scope for improvement.

One of major causes of yield loss is crop competition with weeds. With a push towards reduced pesticide use there is now interest in characters such as allelopathy and competitiveness against weeds as ways to maintain or increase yield. For example, the use of broad, floppy leaves in wheat has been shown to allow it to effectively compete against ryegrass (a major weed) presumably due to better light interception. Increased seedling vigour through the use of larger embryos and longer coleoptiles has also been suggested (Lemerle *et al.*, 2001).

Breeding for Improved Quality

The term quality in crop production has different meanings according to the demands made of the crop products in industry and commerce. It can mean the suitability of the crop product to the technological demands made of it in its extraction or processing (e.g. the milling quality of wheat which is an evaluation of the ease of extraction, and the total amount of flour able to be extracted from the grain). Quality can also mean the extent to which the crop product meets the specifications of commercial demand (e.g. baking quality of wheat; malting quality of barley; taste, size and shape of fruit;

fibre length, diameter and strength of cotton; grain size, colour, taste, protein content, and cooking time in pulses). The term nutritive quality is used to denote the dietary value of the crop product for human or animal consumption. It usually embodies an evaluation of the total carbohydrate, protein and fibre content and the available minerals. It often also includes estimates of the amino acid composition of the protein.

The quality of the crop product can usually be resolved into a small number of components whose variation is correlated with variation in the overall quality of the product. These components are used to measure quality in breeding and to guide selection decisions. Breeders use small-scale tests for evaluating these quality components on small samples from their large numbers of breeding lines.

The level of quality is usually strongly influenced by environmental factors during the growth and ripening of the crop. Often, variation in specific environmental factors can be attributed to variation in the quality of the plant product. Soil nitrogen levels, for example, strongly influence the level of protein in the grain of wheat which in turn influences its baking quality.

However, quality generally exhibits a moderate level of heritability and therefore selection for improved quality is usually successful in bringing about significant improvement. Breeding and selection have resulted in substantial improvement in oil and protein content of the grain of maize and canola and protein content in wheat. In breeding for improved quality crop breeders choose high quality parental lines on the basis of one, or a number, of superior quality components, and hybridise them with the commercial cultivar for quality improvement. In the segregating generations (i.e. F_2 to F_6) from these crosses, selection is carried out for high levels of quality in crossbreds which also bear all the desirable attributes of the commercial variety. When a small number of highly selected, high-quality lines of good agronomic performance have been produced, they are multiplied and further evaluated for equality using methods relevant to commercial practice. In this way a new variety, which is at least equal to the commercial variety in all of its desirable attributes (e.g. high yield, appropriate flowering and ripening times, disease resistance) but superior to it in quality, can be produced.

Selecting for quality in, for example, a wheat breeding program (for bread, biscuit, noodle, steam-bread or flat-bread characteristics), or a lupin breeding program (low anti-nutritional alkaloids, high protein) can consume a very large proportion of the total breeding resources. The samples for quality testing are usually generated from the same randomised, replicated and controlled field trials that are producing yield data, but the cost is significant. Quality characters are very important as producers are targeting diverse markets with strict delivery requirements.

Breeding for Pest Resistance and Tolerance

Breeding for pest resistance and tolerance is a very important activity in crop improvement. Its purpose is to ensure the continued productivity of the crop in situations where pest attack is significant in reducing yields or where the expansion of the crop into new environments is severely limited by the likelihood of pest attack.

The term pest resistance embraces both disease and insect resistance. Crop plants are subject to attack by a wide range of disease organisms such as fungi, bacteria, viruses and mycoplasma. Within particular disease-causing organisms there is usually wide variation in the capacity for, and degree of, infection of the host plant often resulting in characteristic symptoms. This type of variation in micro-organisms is attributed to different races (or strains) of the pathogen. Fungal diseases often attack only a particular plant part (inflorescence, leaf, stem or root) while bacteria, viruses and mycoplasma often attack the plant by penetrating the vascular tissue and producing a systemic infection.

Most crop plants are subject to attack from a wide range of insect pests. Insect feeding activities are often restricted to a specific part of the plant, i.e. root, stem, leaf, or fruit and seed. There are three main ways in which insect infestation of crop plants can cause losses in productivity, and sometimes lowered quality of the product: disease transmission, limitations to normal metabolic activity of the plant, and physical damage leading to reduced physiological efficiency, or plant death. A large number of insects (e.g. aphids) transmit diseases (particularly virus diseases) to plants as a by-product of their dispersal and feeding activities. Many insect pests release toxins into the plant, altering its normal metabolism. This may be expressed by reduced growth and vigour, reduced fertility, proliferation of shoots or even death of the plant. The lucerne bud mite (*Eriopyes medicaginis*), found in many parts of Australia, releases a toxin into the plant which causes stunting and proliferation of shoots, resulting in greatly reduced yields in an infested stand.

Disease Resistance Crop plants have a genetic capacity to minimise the effects of disease attack which is manifested either as resistance or tolerance of the plant to the disease. Resistance includes those mechanisms which prevent or restrict the growth of the disease on the plant. The result of resistance is that the disease organism is found on the host plant in severely limited quantities and its normal growth and reproduction are restricted. The opposite condition to resistance is susceptibility, but the disease-plant relationship can also show varying degrees of resistance or susceptibility. The term tolerance includes those situations which allow some growth and reproduction of the pathogen on the plant but without a significant negative effect on crop yield or quality.

The genetic basis of the plant-disease interaction (usually referred to as the host-pathogen relationship) can be of two general kinds – that controlled by major genes for resistance in the host or by minor genes. However, with fungal pathogens the genetic basis of host-pathogen relationships also involves a corresponding system of genes in the pathogen which interact with the resistance/susceptibility loci in the host in the determination of resistance or susceptibility. This genetic interrelationship between the host and pathogen is quite specific. That is, for every resistance gene in the host there is a corresponding gene in the pathogen which interacts to determine the resistance or susceptibility of the host to the pathogen.

The capacity of the fungal pathogen to overcome the influence of a resistance gene in the host is termed *virulence*; the condition of its inability to infect the host is termed *avirulence*. A fungal pathogen often exists as a range of different types or races with

differing capacities to overcome resistance in the crop plant. An example is given of the relationship of virulence and avirulence genes in the pathogen with resistance and susceptibility genes in the host (Table 4.6). Unless a race of the pathogen bears all the virulence genes corresponding to the number of resistance genes in the host, it is unable to attack the host.

Some fungal pathogens have a capacity to rapidly produce new races with the appropriate virulence gene (or combination of genes) necessary to overcome the resistance in the host. These virulence genes are either already present in the pathogen populations, or arise by mutation. This type of active pathogen creates problems for the crop breeder because new varieties have to be continually bred bearing new sources of resistance. One approach to this problem has been to incorporate two or more different genes for resistance in the one variety so that it will take the pathogen much longer to produce races with corresponding virulence genes. Wheat breeders incorporate multiple sources of resistance genes to wheat stem rust (*Puccinia graminis tritici*) to provide rust-resistant varieties for Australian farmers.

Table 4.6 An example of the genetic relationships of host and pathogen on determining host disease reaction for two resistance loci in the host and the two corresponding virulence loci in the pathogen. Both organisms are considered as homozygous diploids.

Genotype of pathogen	Genotype of host			
	^c R ₁ R ₁ R ₂ R ₂	R ₁ R ₁ ^d r ₂ r ₂	r ₁ r ₁ R ₂ R ₂	r ₁ r ₁ r ₂ r ₂
^a V ₁ v ₁ v ₂ v ₂	susceptible	susceptible	susceptible	susceptible
^b V ₁ V ₁ v ₂ v ₂	resistant	resistant	susceptible	susceptible
v ₁ v ₁ V ₂ V ₂	resistant	susceptible	resistant	susceptible
V ₁ V ₁ V ₂ V ₂	resistant	resistant	resistant	susceptible

^av = virulence allele

^bV = avirulence allele

^cR = resistance allele

^dr = susceptibility

The crop breeder uses a number of different methods for incorporating disease resistance into a variety. The backcross method is commonly used to breed for major gene resistance. With the use of controlled environment facilities in glasshouses or growth rooms, two or three generations can be grown per year. After each backcross the segregating populations are inoculated with the disease and the resistant segregates are selected and crossed again with the recurrent parent. The recurrent parent is usually a commercial variety in which the resistance genes(s) is to be incorporated. In this way new sources of disease resistance can be rapidly incorporated to produce new varieties possessing resistance to current races of the pathogen.

Major genes for resistance and tolerance can also be incorporated into new varieties using the single seed descent or pedigree selection methods for self-pollinated crops and recurrent selection methods for cross-pollinated crops. When resistance is controlled by multiple minor genes these methods can also be used in breeding for resistance; however, the backcross method is not suitable.

Several cases of resistance or tolerance of crop plants to virus attack have been reported. An example is the tolerance of barley (*Hordeum vulgare*) to barley yellow dwarf virus which is conferred by the action of a major gene. An important approach in breeding to minimise losses in productivity due to virus disease is that of increasing the level of tolerance of crop varieties to viruses. The scope for breeding for resistance, either to the entry or multiplication of the virus, or to its insect vectors, appears to be much more limited than that offered in breeding for resistance to fungal pathogens. Narrow-leaf lupin varieties have been bred in Australia with resistance to seed transmission of the seed-borne and aphid-transmitted cucumber mosaic virus (CMV). While the plants themselves remain susceptible they have a much reduced frequency of seed transmission meaning that fewer plants are infected at the start of the next generation and this significantly reduces the chance of an epidemic. Transgenic approaches are being used in lupins to try and produce novel resistance genes that will confer whole-plant resistance to virus replication.

Certain crops are subject to severe attack and losses in productivity from bacterial pathogens. Examples include bacterial wilt (*Corynebacterium insidiosum*) of lucerne (*Medicago sativa*) and bacterial blight (*Xanthomonas campestris* var. *malvacearum*) of cotton (*Gossypium* spp.). Resistance to bacterial infection is genetically controlled and resistance breeding usually involves the incorporation of major genes for resistance against prevalent strains of the pathogen.

Insect Resistance Methods of breeding for plant resistance (or tolerance) to insect attack employ approaches similar to those used for disease resistance and tolerance. The genetics of the insect-host interaction is often generally similar to that described for fungal pathogens and their hosts.

There are three main approaches which plant breeders employ to minimise disease losses from insect attack in crop plants – *antibiosis*, *physical resistance* and *non-preference*. The use of antibiosis involves the incorporation of genes which modify the metabolism of the plant such that the insect through its feeding is adversely affected in growth and/or reproduction. In this way the size of the insect population is restricted and hence losses in productivity are minimised. Breeding for increased physical resistance of plants (e.g. leaves, stems) to attack by sucking or boring insects has been effective in producing varieties with higher resistance to such insects. Non-preference breeding involves the use of genes which modify the morphology or palatability of the plant tissue to deter the insect from feeding and reproducing on the plant. Pubescence (or hairiness) of leaves or stems is a character which is often incorporated to confer non-preference in the host to the insect species.

Breeding for insect resistance is likely to assume a role of increasing significance in future crop production. The deleterious environmental effects of many insecticides plus the capacity of many insect pests to produce resistant biotypes to chemical insecticides demands that more attention be given to breeding for insect resistance and tolerance in crop production. Crops which are particularly attractive to insects and therefore sprayed with insecticide at high frequency, such as cotton, are already the subject of major breeding efforts for increased host-plant resistance, either by conventional means or with the aid of biotechnology.

TECHNIQUES WHICH AID THE CROP BREEDER

While new sources of genetic variability in crop breeding are usually obtained from hybridisation between varieties and crossbreds of the crop species (and more rarely from more distantly related, perhaps wild or weedy, species), there are situations when the crop breeder has to resort to the use of more divergent sources of genetic variability. Several methods are available for this purpose to enable the crop breeder to produce genetic combinations beyond those able to be obtained from normal hybridisation and selection methods. Certain of these methods also enable the crop breeder to gain closer insight into the genetic and evolutionary make-up of commercially important characters. This knowledge can lead to more efficient methods of breeding for improvement in such characters.

Induced Mutation

Methods for inducing mutation have the potential of producing new sources of genetic variability for crop breeding. These methods can be employed when it appears there is little, or no, variability for the character to be improved available within the gene pool of the species. In addition, when a desired character is so closely linked to an undesirable character that there would be little likelihood of obtaining recombinants between them, induced mutation could be used to produce mutants of more favourable expression for the undesirable character, making such genotypes more useful for crop breeding.

The effectiveness of using induced mutation depends on the breeding system of the plant. Its use in self-pollinated plants is likely to be more successful than in cross-pollinated ones. Populations of cross-pollinated plants usually possess stores of genetic variability in the recessive condition and it would not be likely that induced mutation would produce significant amounts of new variability. Induced mutation is potentially useful in the improvement of asexually propagated crop plants. Much of the genetic improvement in present-day horticultural crops can be attributed to the occurrence, selection and propagation of 'sports', or naturally occurring mutations. This indicates that induced mutation could provide further useful variants in these species.

Mutation, in the broadest sense, implies a spontaneous, heritable change in the genetic material at the level of the gene, chromosome or genome. Chromosomal mutation involves such structural changes to chromosomes as translocations, deletions, inversions and duplications, while genome mutation involves loss or addition of one or more chromosomes or chromosome sets (genomes). It also includes the loss or addition of chromosome arms (see below).

There are three broad categories of mutation-inducing agents (called mutagens) – ionising radiation, ultraviolet radiation, and chemical mutagens. Ionising radiation (e.g. x-rays, gamma rays and alpha, beta and other fast-moving particles) cause both gene mutation and chromosomal breakage. Their mutagenic effect is probably due both to direct 'damage' of the chromosome and to ionisation which produces chemical

changes in the cell. Ultraviolet radiation has low penetrability and is used mainly for irradiation of pollen prior to its use in fertilisation. Chemical mutagens generally produce a greater ratio of point (gene) to structural mutations than radiation. The most commonly used chemical mutagens, hydroxylamine, nitrous acid, and alkylating agents (*e.g.* ethylmethane-sulphonate, or EMS) modify the chemical composition of DNA to produce a wide range of mutations in higher plants.

Induced mutation techniques usually involve treating the seed with radiation or chemical mutagens under specified conditions of mutagen dose or concentration together with close control of the experimental conditions during treatment. The rate of induced mutation is dependent on the maintenance of precise conditions of temperature, oxygen concentration, pH, and moisture content of seed during the treatment period. Pollen irradiation is carried out under precise conditions of temperature and humidity. The irradiated pollen is then used in hybridisation, and mutants are sought in progeny arising from this hybridisation.

Most of the induced mutations in plants are recessive and therefore can only be detected after segregation in the second generation after treatment. A scheme for using induced mutations and selecting for mutants in crop breeding is shown in Figure 4.17. The terminology for generation number in mutation studies is as follows: M_1 denotes the generation of mutagen treatment (i.e. the plants arising from treated seed or from hybridisation using irradiated pollen); M_2 denotes the following generation in which (recessive) mutations would segregate in the double recessive condition and M_3 , M_4 ...subsequent generations.

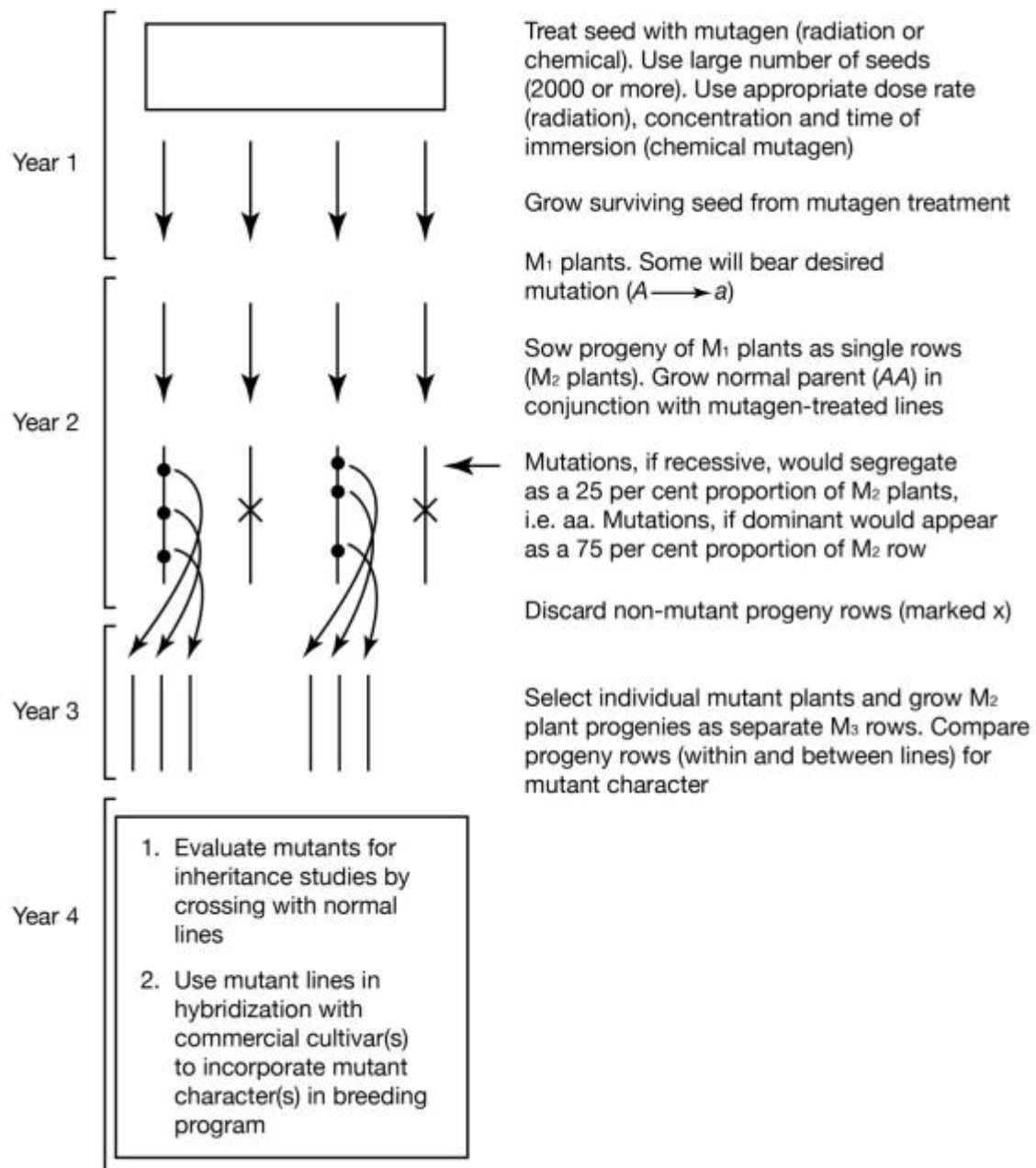


Figure 4.17 Outline of a scheme for producing mutants for crop breeding

While there are examples of induced mutation having produced improvements of commercial significance in certain crop plants (e.g. stiff-strawed, lodging-resistant mutants in barley, and increased yield in peanuts), its contribution to crop improvement has not, overall, been substantial. It appears that within the gene pool of most crop species there are still adequate amounts of genetic variability for crop improvement for characters of commercial significance. Induced mutation would therefore be of significance only when genetic variability becomes limited from natural sources, or when potentially useful genes are very closely linked to deleterious genes in the crop plant or its related species. When lupins were first bred in Australia, for example, various domestication characters under simple genetic control were either found as natural mutants, or were induced artificially (Gladstones *et al.*, 1998).

Cytogenetics

Cytogenetics is concerned with the study of the physical appearance (e.g. morphology, number) and behaviour (e.g. pairing, movement) of chromosomes in conjunction with genetic interpretation of the causes and consequences of variation in their appearance and behaviour.

Present-day crop plants can be hybridised with the wild and weed relatives from which they were derived, and in these hybrids genetic recombination takes place between the chromosomes of the crop plant and the wild or weed species. However, many crop plants originate from more distantly related species which can be hybridised with them but whose chromosomes show little or no pairing with those of the crop plant. Cytogenetic methods can help provide access to this source of genetic variability.

In plant breeding, chromosomes can be manipulated at several levels: genome multiplication, genome addition, whole chromosome, and chromosome segment. In addition, recombination between chromosomes can be manipulated. The exploitation of haploidy is another (powerful) case and is discussed later.

Induced Polyploidy Crop breeders can alter the ploidy level (the number of complete chromosome sets) of a plant, either to increase it (polyploidy) or to decrease it to half that of normal (haploidy – discussed below). The simplest ploidy level is diploid where the plant has two copies of each chromosome but many crop plants are polyploids. Wheat, for example, is a hexaploid consisting of three ancestral genomes (A, B and D) with a base number of seven. Therefore, the total chromosome number in wheat (AABBDD) is 42. In genetic literature this information is summarised as:

$$6x = 2n = 42.$$

Triploid watermelons, which are high yielding and seedless, triploid sugar beet (*Beta vulgaris*) which is generally of higher sugar yield than the diploid, and tetraploid red clover (*Trifolium pratense*), which has higher forage yield than the diploid, are three examples of commercially successful induced polyploids.

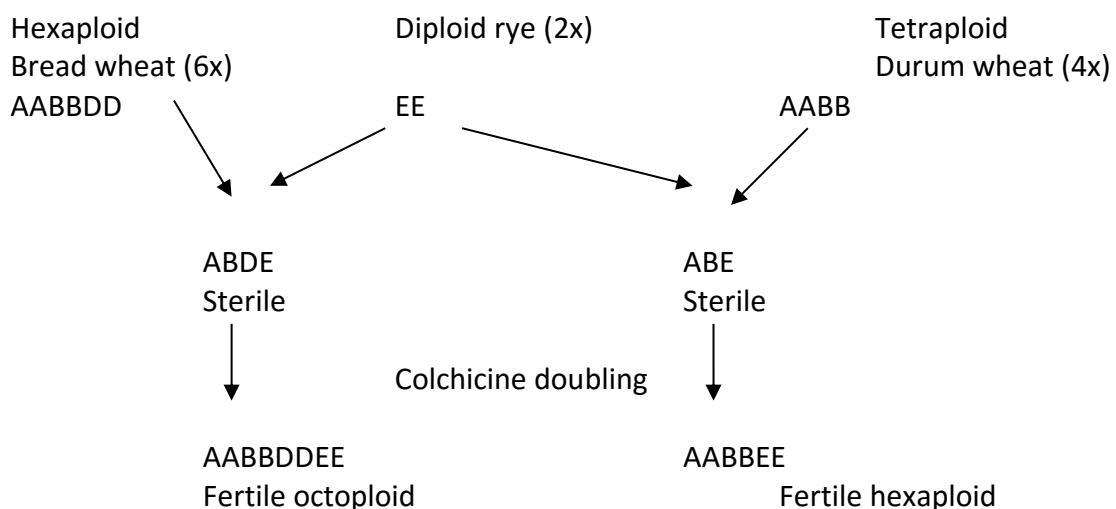
The technique most commonly used in inducing polyploidy involves immersion of germinating seeds or saturation of apical meristems with colchicine, an alkylating agent derived from the bulb of the autumn crocus (*Colchicum autumnale*). Colchicine is used in concentrations ranging from 0.1 to 0.5% and treatment times ranging from 4 to 24 hours. The action of colchicine is to inhibit cell wall formation between the anaphase groups of chromosomes at mitosis and instead of two daughter cells with the diploid (2n) chromosome number the undivided cell will have the tetraploid (4n) number of chromosomes. Cells of this constitution, if they occur as a significant proportion of the cells in the meristematic region of the shoot, can give rise to tetraploid tissue and, in turn, to tetraploid shoots. Verification of the induction of tetraploidy can be carried out from chromosome counts at metaphase of meiosis of pollen mother cells in the developing flowers of these shoots or at metaphase of mitosis in root tips of germinated seeds from the next generation. The guard cells in the leaf epidermis are also larger with increased ploidy level.

The most important role of induced polyploidy in crop breeding is as a technique in distant hybridisation programs between certain crop plants and their related species. It has been used as a means of increasing the frequency of successful hybridisations between the crop plant and a related species. For instance the frequency of successful hybridisations between cultivated tomato (*Lycopersicon esculentum*) and one of its related wild species (*Lycopersicon peruvianum*) is significantly increased if the cultivated tomato, as the female parent, is tetraploid rather than diploid.

Whole Genome Addition The crop plant can be hybridised with the related species and if there is no pairing between the two chromosome sets the resultant hybrid can be treated with colchicine to double the chromosome number giving a fertile hybrid. The genome(s) of the related species have, in effect, been added to those of the crop plant. The scope is limited to adding whole genomes of related species to the crop plant to produce commercially acceptable crop types. An example of a hybrid of this type which has achieved notable commercial acceptance is Triticale, which is the addition of the genome of cereal rye to hexaploid wheat to produce octoploid triticale or to tetraploid wheat to produce hexaploid triticale. Hexaploid triticale is grown commercially in a number of countries throughout the world and its grain is used as an animal feed (see Example 4.1).

Example 4.1 The routes to Triticale synthesis – an example of a synthetic crop species produced by man.

Diploid rye is hybridised with either hexaploid bread wheat or tetraploid durum wheat. The resulting hybrids have an odd chromosome number and are sterile. Fertility is restored by doubling the chromosome number with colchicine to produce either octaploid or hexaploid triticale. Both wheat species and rye have a basic chromosome number of 7. The floral meristems are doubled before flowering so that the doubled ears contain fertile seeds.



Whole Chromosome Transfer Individual whole chromosome transfer can be made from a related species to the crop plant by backcrossing using the crop plant as the

recurrent parent. The aim of selection is to retain a particular chromosome from the related species on the basis of chromosome number and morphology and its particular modification of the phenotype of the crop plant. In this way a chromosome from a related species can be added to the chromosome complement of the crop plant. As a single chromosome addition it is referred to as a *monosomic addition* line; as the homologous pair it is a *disomic addition* line. The chromosome addition line can then be used for substituting a pair of homologous chromosomes of the crop plant with the added chromosome pair from the related species and is referred to as a *chromosome substitution* line. However, the main value of whole chromosome transfer from related species to the crop plant is for detecting the chromosomal location of useful genes which can be further manipulated. Two methods, induced translocation and induced chromosome pairing, have been used for this purpose. Induced chromosome pairing has been demonstrated in wheat where, in hybrids between wheat and certain of its related species, the removal or suppression of pairing-inhibitor genes allows pairing to take place between the chromosomes of wheat and the related species.

Chromosome Segment Transfer Considerable success has been achieved in crop improvement through the use of methods which enable transfer to be made of segments of chromosomes of related species onto chromosomes of the crop plant. These methods have been used mainly for transferring major genes for disease resistance, from the related species to the crop plant. The transfer is achieved by irradiating the hybrid (or the chromosome addition or substitution line) between the related species and the crop plant with x-rays or gamma rays to produce random breakage and rejoining of its chromosomes. In the progeny of the irradiated plant it is possible to select genotypes which contain the full chromosome complement of the crop plant but with one chromosome bearing a segment of the related species' chromosome possessing the desired gene. This method of effecting genetic exchange between chromosomes through breakage and rejoining is termed *induced translocation*. The wheat variety Eagle in 1969 possessed a translocation from the related species, *Agropyron elongatum*, which carried a gene conferring resistance to wheat stem rust (*Puccinia graminis tritici*).

Recombinational Another approach to transferring useful genes from chromosomes of related species to those of the crop plant, when there is no pairing between their chromosomes, is the manipulation of genes restricting this pairing behaviour. In wheat it has been demonstrated that certain genes regulate the specificity of chromosome pairing within the plant to prevent pairing between genetically similar chromosomes in its three genomes (A, B and D). These genes also inhibit pairing between wheat chromosomes and those of certain of its related species in hybrids with them. However, by the removal or suppression of pairing-inhibitor genes it is possible to effect pairing and genetic recombination between wheat chromosomes and those of many of its related species. Details of cytogenetic techniques are provided in genetics texts on the subject (e.g. Appels *et al.*, 1998).

TECHNIQUES USED IN PLANT BIOTECHNOLOGY

Techniques are now available to rapidly sequence DNA so that the genetic code of a whole genome can be obtained – the Human Genome Project has been a striking success. The experimental plant *Arabidopsis thaliana* is the first plant to be completely sequenced as has rice (*Oryza sativa*) and other plants are under study (*New Scientist*, 16 December 2000). Large-scale DNA sequencing and the analysis of the huge amounts of data generated (genomics and informatics) has great potential for improving the speed and efficiency of plant breeding, and for tackling difficult or novel characters.

Progress in plant breeding is highly dependent on developments in related disciplines, such as biochemistry, plant physiology and plant pathology. A detailed knowledge of the DNA sequence of a plant's genome enables more rapid progress in these disciplines, and a greater understanding of the basic processes occurring in plants. A comprehensive treatment of DNA technologies is beyond the scope of this chapter.

Modern biotechnology has become possible due to an increased understanding of gene structure and function at the cellular and molecular level – an understanding that is advancing at a rapid rate. The main techniques associated with biotechnology (or 'genetic engineering') in plant breeding are: recombinant DNA manipulation and genetic transformation, DNA-based (molecular) markers, tissue culture, protoplast manipulation, *in vitro* selection and hybridisation, and the use of monoclonal antibodies. Undoubtedly others will be added as knowledge increases.

DNA Markers The use of DNA, or molecular, markers will have the most significant effect on all plant breeding programs. We have already seen how the breeder has to try and improve or maintain a range of 'difficult' characters – those greatly influenced by the environment, or those time-consuming or expensive to measure. Such characters can be handled a lot more efficiently if closely-linked DNA markers are available for the genes controlling the character.

The most common and useful DNA markers are the ones detected by the *polymerase chain reaction* (PCR) which amplifies short pieces of DNA identified by specific end sequences. PCR technology is rapid, cheap, unambiguous, allows detection of variation in DNA down to the single base-pair level, and can be carried out on a large number of individuals.

DNA markers are based on differences in the actual DNA sequence. The piece of DNA in question may or may not be part of a gene with a recognised product (such as an enzyme, a structural protein, or a change in a morphological character). When DNA is 'cut' with restriction enzymes, the cut sites occur in different places and consequently the fragments of DNA produced are of different lengths. These pieces can be amplified (if required), separated by electrophoresis, visualised, sized and quantified. The fragment sizes vary between species, varieties and individuals (especially in outbreeding species or in hybrids). Once some markers are identified, they can be located on chromosomes in relation to themselves and other known genes, such as morphological markers, isozymes and disease resistance genes. The markers are then available as a fine-detail map of the plant's genome – the more markers, the finer and

the more useful the map. This map can then be used to locate other major genes and, perhaps more importantly, to locate genes that contribute to quantitative traits such as yield. These quantitative trait loci (QTLs) can then be tracked by monitoring a closely-linked marker during breeding. Molecular markers are very powerful. Suppose that one marker was closely linked to a QTL that was responsible for, say, 15% of yield in the major current varieties of wheat: being able to guarantee the presence of that gene in all selected progeny from a cross by using the marker would boost immensely the efficiency of the breeding program. The process of employing molecular markers is called marker-assisted selection (MAS) (Example 4.3).

Marker maps are available for most important species, including wheat, barley, maize, canola, rice, lettuce, soybean, lentils, tomato, and potato. Progress is rapid in the *Triticeae* because of the availability of numerous cytogenetic stocks and because of the homoeology of chromosomes from different species. Homoeology results in a high level of cross-hybridisation; for example, some markers from barley and a wild relative of wheat, *Triticum tauschii*, are also found in wheat.

Example 4.2 Marker-assisted selection – a wheat example

New wheat varieties for the Australian state of South Australia and parts of Victoria must be resistant to cereal cyst nematode (CCN) to have widespread commercial acceptability. Resistance to CCN (CCN-R) is known to be controlled by a single gene. However, screening for CCN-R in breeding populations of segregating wheat plants is fraught with difficulty. A field infestation of the nematode cannot be guaranteed. Plants have to be carefully examined under the microscope. A PCR-based DNA marker has been developed (originally it was an RFLP-based marker) which allows the breeder to screen segregating populations (a number of progeny produced from a CCN-R x CCN-Susceptible cross). DNA is extracted from one leaf of each individual seedling so that the seedling remains alive and capable of being grown-on to produce seed. The DNA samples are run through a thermocycling machine with the PCR reaction mixture and the PCR primers that are specific for the DNA marker for CCN-R. The resultant DNA is visualised in lanes or tracks on an electrophoretic gel or as a colour reaction in the well of a micro-titre plate. The positive individuals that have the DNA marker gene will also have the CCN-R gene. The breeder can then discard the susceptibles and grow-up the CCN-R lines and continue selection for other characters.

The tomato map is well advanced and its value was dramatically demonstrated when a gene for resistance to a virus (*Tm-2*) from a wild tomato species was backcrossed into a cultivated line. By monitoring closely flanking markers, the introgressed chromosome segment was so small after only two generations that to achieve the same result conventionally would have taken about 100 generations. Markers have been mapped close to very important QTLs in tomato, such a water-use efficiency, fruit soluble solids, fruit mass and pH.

Meristem Culture, Clonal Multiplication and Disease Elimination These techniques are aspects of the same technology – micropropagation. Tissue culture is widely used to multiply a range of cultivated species (e.g. sugar cane, bananas and pine trees). It is particularly useful for overcoming international quarantine restrictions, and for

speeding up the breeding of perennial and woody plants. The culturing of 'clean' meristem tissue allows disease-free (particularly virus-free) varieties to be rapidly produced, especially in clonally propagated crops (e.g. grapes, citrus and strawberries). These applications are most effective when genetic changes induced during culture can be kept to a minimum (Smith and Drew, 1990).

Somaclonal Variation Somaclonal variation is genetic change that occurs as a direct result of the stress of tissue culture. It is diverse in character and varies dramatically in frequency and type with species and genotype (Evans and Sharp, 1986). Somaclonal variation may offer a different spectrum of genetic changes to spontaneous or induced mutations (for example, from the effects of ionising radiation). However, unless variant production is linked to some efficient selection procedure (preferably at the cellular level) then the system may be too unwieldy. Somaclonal variation can occur simultaneously in several characters. Consequently, much conventional clean-up breeding is required to remove undesirable gene combinations.

Induced Haploidy Haploidy occurs at a low frequency in all crop plant populations. Haploids are individuals with half the chromosome number of the normal diploid or polyploid from which they are derived. Consequently, they possess only one chromosome of each homologous pair of their parental source. In polyploid species the haploid is usually viable but sterile, while in diploids it often dies very early in its development.

Haploidy has been induced in many crop plants, both monocotyledons and dicotyledons, through the culture of immature microspores on specialised growth media under sterile conditions. In higher plants the young male gamete, or microspore, which usually gives rise to the pollen grain, also has the potency to develop callus tissue or plantlets according to its nutritional environment. Because of the haploid condition of the microspore the vegetative growth arising from it is also haploid and the induced plantlet will give rise to a haploid plant. In some species the fertile diploid condition is spontaneously restored in some of the regenerant plantlets produced from tissue culture, removing the need for induced chromosome doubling using colchicine.

The technique of induced haploidy used in conjunction with that of chromosome doubling of haploid plants offers the crop breeder scope for greatly reducing the time normally taken to reach homozygosity in the breeding programs of self-pollinated species. The induction of haploidy in the pollen of heterozygous plants and the induction of a doubling of chromosome number in the haploids enables homozygosity in the segregates to be reached in one generation rather than approximately six generations (F_2 to F_7) with normal sexual reproduction (Figure 4.18).

The use of haploidy in cross-pollinated crops is more restricted since the resultant dihaploids are, by definition, 100% homozygous – a deleterious condition in a species where heterozygosity is the norm. Induced haploidy holds considerable potential in shortening the length of time for the breeding of new crop varieties but two aspects must be improved: (a) a more consistent and higher frequency of haploid induction from a broad range of genotypes; and (b) more reliable chromosome doubling of haploid plants.

Notwithstanding these difficulties, microspore culture is an excellent tool for speeding up gene transfer by conventional hybridisation and backcrossing, where only a few fertile regenerants are required at each generation. The technique becomes even more powerful when coupled with the use of linked DNA markers (described later) because of the increased speed and precision of the selection process (see Example 4.3).

Example 4.3 The use of introgression, backcrossing and dihaploidy to produce the Australian commercial two-row barley variety *Tantangarra*.

The fungal disease scald (*Rynchosporium secalis*) is a potentially damaging disease in barley. Long-term research at CSIRO in Canberra identified scald resistance genes in *Hordeum spontaneum*, a wild relative of barley. These genes were successfully transferred to cultivated barley (variety *Clipper*) by way of artificial hybrids between the two species and backcrossing to *Clipper*. The genes were then located to individual chromosomes and identified as being linked to polymorphic enzyme loci (isozymes) (Abbott *et al.*, 1992).

A *Clipper* backcross line containing one of the scald-resistance genes was crossed with variety *Skiff* (good yield, good adaptation) to produce F₁ and then F₂ plants. Anthers from F₂ plants were placed in tissue culture and doubled haploids (DH) were produced (either spontaneously doubled or doubled with colchicine)(Figure 4.19). The set of DH lines were rapidly multiplied and tested in field trials to eliminate scald-susceptible ones. Finally, the best line with scald resistance was released as *Tantangarra* (Luckett and Smithard 1992). The variety found wide acceptance as a high-yielding feed barley.

In Vitro Selection This technology allows the selection of cells in tissue culture that are resistant to, or tolerant of, specific chemical hazards, such as fungal phytotoxins, herbicides, acidity, salinity, or heavy-metal toxicity. Once these cell lines are produced, there remains the problem of whole-plant regeneration (common to much biotechnology) and whether the observed resistance is genetically determined and therefore inherited.

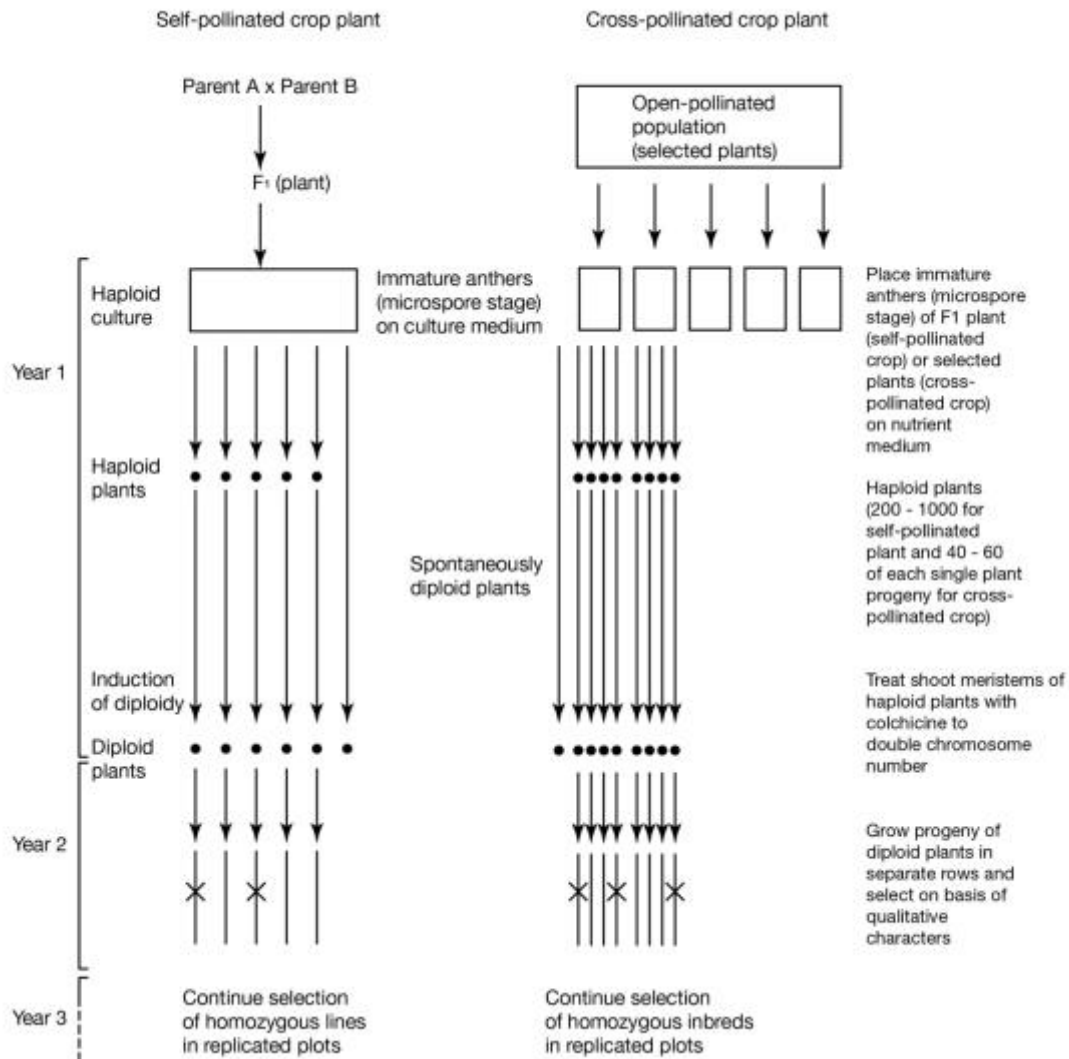


Figure 4.18 Procedure for using induced haploidy in crop breeding to achieve (a) early selection of homozygous lines in a self-pollinating crop, and (b) rapid production of inbreds as parents for breeding in a cross-pollinating crop

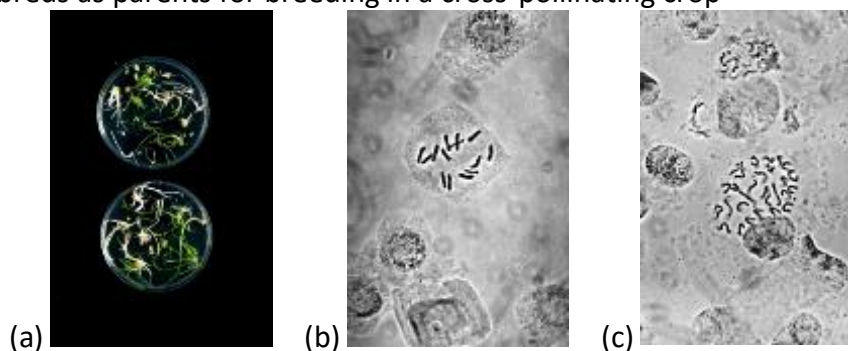


Figure 4.19 (a) Green and albino regenerant barley plantlets germinating on defined tissue culture media in sterile petri dishes from embryos produced by in vitro anther culture. Albino plantlets are non-viable.

(b) Mitotic root-tip squash from a single cell of a spontaneously doubled Barley plant ($2n=2x=14$) produced from anther culture (as stained and viewed under a microscope). The individual chromosomes are visible as dark, condensed rods.

(c) A second mitotic root-tip squash from a colchicine-treated barley

Regenerant produced from anther culture. The colchicine has created a tetraploid ($2n=4x=28$). Contrast with the (b).

In Vitro Hybridisation Somatic cells (usually protoplasts) of a crop and a distantly related species can often be induced to fuse in tissue culture. This method is a useful tool for the transfer of nuclear or cytoplasmic genetic material between genotypes that are not normally sexually compatible. Desirable traits can be transferred even when the precise genes remain undefined. It has been used widely in the *Solanaceae* and *Cruciferae*, and also recently with lucerne, medics and rice.

DNA Manipulation The manipulation of DNA is central to the genetic engineering aspect of biotechnology. There are several ways that geneticists identify and then isolate a gene of interest. The primary route is to identify a protein (or its precursor, an RNA) which is associated with a certain plant characteristic of interest. The protein is first isolated and purified. The amino-acid sequence allows several possible DNA coding sequences to be proposed, due to the redundancy of the triplet code. One of the DNA sequences is then synthesised, or more usually a portion of it. The resultant oligonucleotide is then used to 'probe' (i.e. test by DNA hybridisation) a genomic library of the organism's DNA maintained in some microorganism (usually *Escherichia coli*, but yeast is also used) to find which fragment in the library has the full original gene. The gene can then be located in the genome, cloned (multiplied in *E. coli*) and ultimately sequenced to understand its structure and function. With appropriate modifications the gene is then available to be inserted into another, usually unrelated, plant species requiring improvement.

Another way to track down a gene is to use a *transposon* ('jumping gene') of known DNA sequence to allow 'transposon tagging'. Essentially, the transposon is allowed to cause mutations by moving about the genome until the plant character of interest is affected. The transposon is then assumed to have inserted itself in a gene associated with that character and to have disrupted its normal function. A DNA library is then made of the genome, and the fragments which hybridise with the transposon sequence will be flanked by the gene of interest. Once isolated, investigation of the gene can continue as outlined above.

Biotechnology has stimulated a massive increase in our knowledge of gene structure and function. It is now routine to couple specific protein coding regions of DNA with control sequences from different species to allow tissue-specific or stimulus-specific gene expression. However, many fundamental problems remain. Most of the characters of plant breeding relevance are multigenic traits, with the genes of small effect scattered throughout the genome and exhibiting a myriad of pleiotropic or epistatic effects. Simply finding the genes is a major problem.

As knowledge of DNA sequences and genes in other species increases, it becomes routine to search the genome of the target crop species for similar genes. This approach, the field of *functional genomics*, allows scientists to rapidly home-in on genes of interest, to predict likely gene function by sequence comparison with previously-studied genes, and also design completely novel genes.

Once a foreign gene is inserted, its location, copy number, and orientation will affect its performance, and evidence is mounting that this may be a major problem. The inserted gene is disrupting a complex interconnected system of thousands of genes, so the results can be unpredictable.

Gene Shears and Antisense RNA This technology adopts a different approach – to remove the effect of an undesirable gene in a crop plant via biotechnology, rather than to add a new one. The technology depends upon RNA molecules (ribozymes) that themselves function as ‘enzymes’ and which are capable of cutting or splicing other RNA molecules and thereby disrupting their function. The ribozyme can be engineered to cut any m-RNA of known sequence; it can therefore be designed to interfere with virus replication, for example, or switch-off a gene with an undesirable phenotype.

A similar approach to gene shears is being used with an undesirable gene that is responsible for fruit softening in ripe tomatoes. The effect of the gene can be overcome by chemical treatment with a polyamine after harvest but the more elegant solution is to identify and disable the gene responsible. One approach is to produce what is called antisense DNA to the gene to be disabled. The normal gene and its inserted antisense cousin both produce m-RNA but because the two strands are exactly complementary they bind together to produce non-functional molecules. The function of the normal gene is then largely eliminated (Day, 1989). The gene shears system outlined above could also be used to achieve a similar result. The outcome is that the neutralisation of this gene results in ripe tomatoes that stay firm for much longer and have a greatly increased shelf life. Conventional tomato breeding has made progress in this area by the use of non-ripening and ripening-inhibitor genes found in cultivated genotypes.

Genetic Transformation Once a gene has been isolated, cloned and modified, the task is then to achieve stable incorporation into the genome of a suitable recipient species (initially usually a ‘model’ plant species such as *Arabidopsis* or tobacco). This transformation can be attempted in a number of ways (Perani *et al.*, 1986; Potrykus, 1991):

- using a biological vector such as *Agrobacterium* or cauliflower mosaic virus to carry the ‘hitch-hiking’ foreign DNA into the plant. This technology is well understood for dicotyledons, but the two species of *Agrobacterium* will not generally infect monocots. However, extensive research has induced *Agrobacterium* into infecting monocots and success has been reported in at least asparagus and onion;
- naked DNA uptake by protoplasts or some other cell without a cell wall, such as a pollen tube, which may be facilitated by temporary membrane disruption caused by treatment with polyethylene glycol (PEG), the application of a rapid electric current (electroporation), or sonication;
- by physically inserting the DNA. One method is to use a very fine glass needle to inject the nucleus of protoplasts with a DNA suspension, using either pressure or electrophoresis to move the DNA (micro-injection). Another method inserts the DNA ballistically on the surface of very small, inert carrier particles, such as tungsten pellets (biolistics).

The major food crops (mainly monocotyledons) are not as amenable to these techniques as many dicotyledons, since fertile plants cannot easily be regenerated from protoplasts. However, progress has been made with rice, wheat and barley.

Antibodies Highly specific monoclonal antibodies can now be produced by tumour cells growing in culture. The lifespan and production capacity of such cultures is virtually unlimited. The antibodies bind to certain specific protein molecules, thereby providing qualitative (and possibly quantitative) measurements. This powerful technique, coupled with its visualisation technology ELISA (enzyme-linked immunosorbent assay), can be used to detect, in plants, protein from such foreign sources as viruses, fungi, bacteria, mycoplasmas, mycotoxins and hormones (Reddy *et al.*, 1988). In addition, antibodies can reveal the presence (or absence) of specific genes by illuminating the gene's indirect protein product. These detection methods can be used in a breeding program, for example, to detect for disease resistance or certain quality characters.

DNA Diagnostic Probes These probes are useful in a similar way to antibodies. They hybridise with DNA of a corresponding sequence, and then when coupled with appropriate indicator molecules that are radioactive or produce a coloured reaction, the hybridisation can be visualised. This allows a qualitative (if not quantitative) test for the presence of particular DNA, be it from a closely related species or genotype with which hybridisation has occurred, or from a pathogenic organism such as a virus, bacterium or fungus. The polymerase chain reaction (PCR) is often employed in this context because of its ability to chemically amplify very small quantities of DNA.

Examples of Biotechnology Application A wide range of genes have been cloned by DNA manipulation from a host of different organisms. Many of them could eventually be of agricultural significance once modified, equipped with the necessary controls, and stably inserted in an appropriate location in a recipient species. The list will continue to expand for a bewildering range of proteins and enzymes.

A range of gene control sequences have been isolated and characterised, and they can be coupled to structural genes to give a useful functional unit. Controlling sequences respond to stimuli such as light, zinc concentration, wounding, tissue location, and low oxygen concentration. This allows novel genes to be constructed; for example, insect toxin genes that are only expressed when tissue damage occurs from insect feeding, or a herbicide resistance gene which is activated by a topical zinc spray.

The modification of plant incompatibility systems has great potential, as the following example illustrates. A gene promoter specific to anther tissue was discovered and joined to a ribonuclease gene. The new gene was then inserted into tomato, tobacco and canola using *Agrobacterium*. It prevented normal anther development, and the transgenic plants were male-sterile. Such manipulation allows the economic production of hybrid F₁ seed in a normally self-pollinating crop (Peacock, 1990).

The prevention of plant disease has been addressed by biotechnology in at least two ways. The first is to engineer a 'disabled' viral genome to trigger a response in the 'infected' plant but cause no damage. This preconditioning allows the plant to resist subsequent infection much more effectively. The second approach is to produce

synthetic resistance genes which employ a decoy satellite RNA to mop up the RNA polymerase produced by a virus, without which it cannot replicate (Courtice, 1987).

The nutritional quality of crops can be improved by biotechnology so as to benefit human health and animal production. An increased sulfur-containing amino acid concentration in cereal protein would be beneficial to humans and stock, for example, by inserting pea storage-protein genes into wheat.

The first products of plant biotechnology are being grown on farms. Cotton plants engineered to contain *Bt* toxin genes from the bacterium *Bacillus thuringiensis* for insect resistance, have been developed by CSIRO and have been commercially-grown in Australia since 1996. The protein produced by the *Bt* gene is specific to *Lepidoptera* insect pests that graze on the plants. Cotton varieties are also available that contain transgenes for herbicide resistance. A pea-weevil resistance gene has been inserted into field peas thereby removing the need for insecticide sprays to control this pest.

More fundamental work on basic plant processes will be required to support long-term progress in the application of biotechnology to plant breeding, particularly in biochemistry, physiology and pathology. These basic processes include: the control of vernalisation, factors limiting photosynthesis, nitrogen fixation, differentiation, incompatibility systems, photoregulation, stress response, wound response, and response to pathogen invasion. Functional genomics will play a major role in these areas.

The production of transgenic plants has raised considerable ethical and ecological issues which are the subject of vigorous public debate. While the future of transgenics is still unclear, the increasing role of DNA marker technology to improve conventional breeding technology is assured. Transgenics are likely to be much more widely acceptable when the transgene controls a 'consumer-benefit trait' rather than a 'producer-benefit trait'. Engineered crop herbicide resistance is a producer-benefit trait which raises numerous serious issues – not least the sustainability of the farming system. A consumer-benefit trait, especially one with a public health or medical benefit, is likely to be rapidly and widely adopted.

The relationship between conventional and biotechnological methods in plant breeding in terms of the flow of hereditary materials is shown in Figure 4.20.

VARIETY EVALUATION AND RELEASE

Before an advanced crossbred of a crop can be released for commercial production it must undergo rigorous evaluation for yield, quality, and agronomic characters (e.g. ease of harvesting, non-shattering, lodging resistance, and disease resistance). The evaluation of the commercial potential of advanced crossbreeds of a crop is usually performed in replicated experimental plots over a range of sites representing the environments for which the variety is being bred and using normal commercial practice. This evaluation is usually carried out over four to five years, or longer, to gain an accurate estimate of average seasonal performance. In conjunction with yield evaluation it is usual to conduct quality evaluation of the crop product using small-

scale commercial evaluation methods closely related to commercial practice. This corresponds to Phases 3 and 4 of the breeding program.

The crop breeder needs a detailed knowledge of the nature and magnitude of genotype-by-environment interaction (G x E) for any new variety approaching commercial release. The level of G x E will influence the growing areas for which the variety will be recommended, and will provide information regarding situations where the variety will not perform well, e.g. avoid late sowings or only suitable for southern New South Wales.

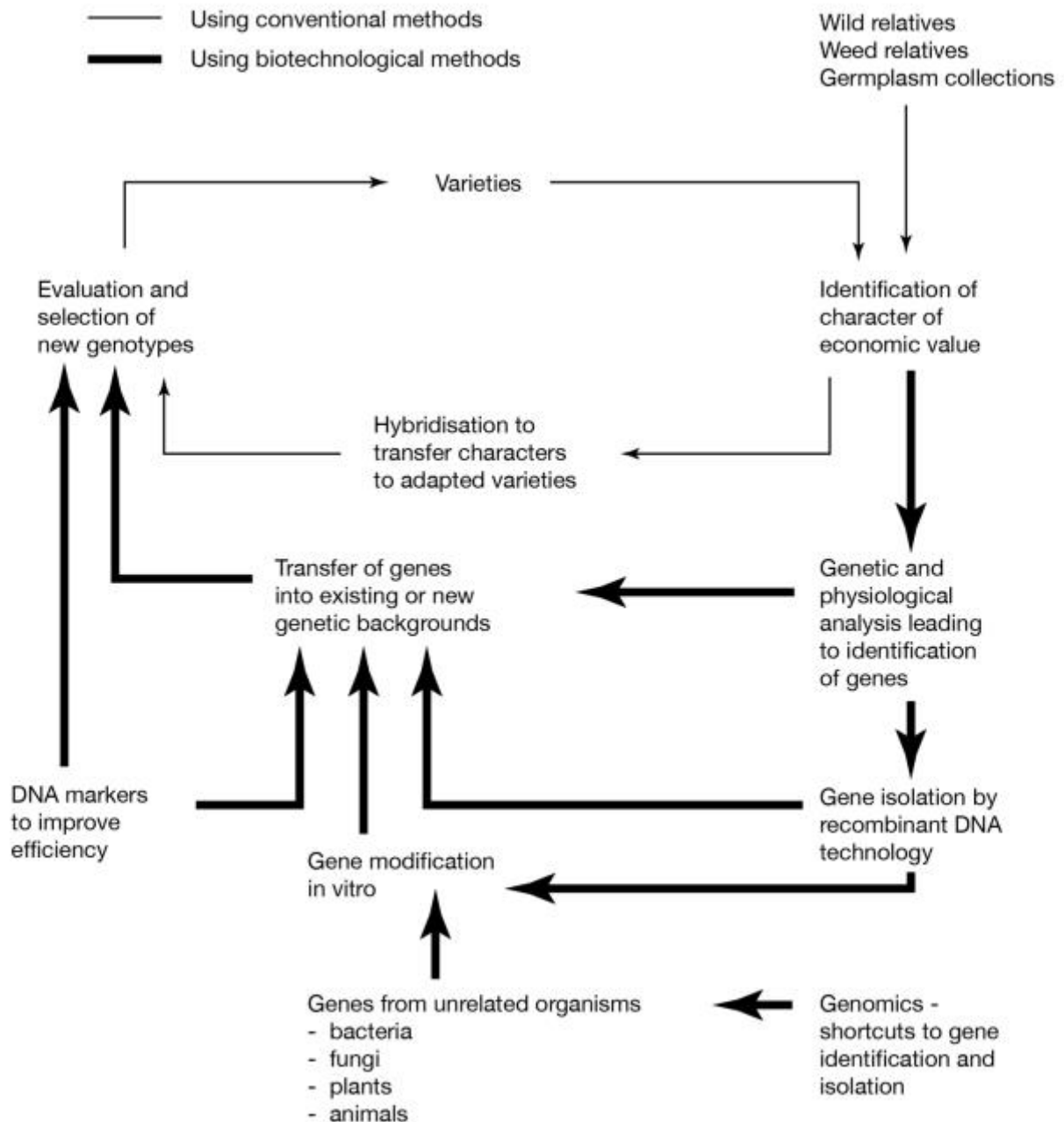


Figure 4.20 The flow of hereditary material in plant breeding

When a crossbred has performed satisfactorily and sufficient seed is available, it is named and released for commercial production. The release of a new crop variety entails a detailed description being made of its morphological characters, for both the plant and harvestable product (e.g. grain, and the characteristics of commercial significance such as quality). In the case of disease resistance the description embodies a statement of the diseases, and the particular races of the disease to which the variety is resistant. When it is known, the particular gene designations for resistance possessed by the variety are also stated. The description also includes a statement on the pedigree of the variety. At release the variety is given a name which is carefully chosen so that it cannot be confused with existing or earlier varieties of the crop. Particular breeding programs often use a naming theme to identify their varieties (e.g. wheat varieties bred at Wagga Wagga, NSW, are named after birds).

Plant Breeder's Rights

In 1987 the Australian Government enacted Plant Variety Rights legislation and in 1994 amended this with the Plant Breeder's Rights (PBR) Act. PBR is an extension of the concept of allowing ownership of an invention and is similar to copyright legislation. PBR is a limited form of proprietary ownership for up to 20 years to prevent others using a new plant variety without the owner's permission, except for their own use or for further research. However, under PBR, growers are able to retain seed from self-pollinating crops for future sowings. The aim of PBR is to protect breeders' intellectual property, stimulate private-sector breeding in Australia, and to ensure that Australian farmers have access to suitable varieties that are registered overseas.

New varieties submitted for PBR registration must arise from selective breeding, must be uniform and stable over generations, and be distinguishable from all other varieties. Seed, cuttings, or other propagules of a variety protected by PBR may not be freely sold. In this way, the PBR holder (who may, or may not, be the original breeder or their employer) is the only person permitted to distribute the variety and charge royalties for the duration of the legal protection. There is an increasing world-wide trend to attempt to patent biological material, including plant varieties, and especially the products of biotechnology. Patents provide much tighter ownership rights than PBR, but there is considerable concern about the moral difficulties and practical consequences of such a course. Nearly all new plant varieties released in Australia are now protected by PBR. Older, freely-available 'public' varieties are rare.

Pure Seed Production

It is important that the genetic identity of the variety be maintained after release and throughout its commercial life so that its performance in all characters accords with that specified as justification for its release as an improved variety. For this reason the production and maintenance of pure seed stocks of the variety as a continuing source of seed for commercial sowing is an important activity in crop production. Pure seed production is organised at a number of levels concerned with both the assurance of genetic identity and providing sufficient volumes of seed for commercial plantings.

The initial level of seed multiplication is *Breeder's Seed* which is seed or vegetatively propagated material produced by the breeder, or his institution, which is exactly true to the variety type. Breeder's seed gives rise to *Pre-basic* and then *Basic Seed* which is usually produced by the research organisation which bred the variety. In its production, strict precautions are taken to ensure that the pre-basic and basic seed is true to its genetic identity. This seed is usually distributed to commercial seed growers or sold to commercial partners who grow it to produce *Certified Seed*, usually for two generations. Certified seed must meet strict genetic purity standards and be free of disease and weed seeds. The activities of producing certified seed from breeder's seed are continuous; they ensure continued purity of the variety in its commercial production until it falls from favour and is replaced by newer varieties.

CROP PLANT INTRODUCTION AND BREEDING IN AUSTRALIA

Crop Plant Introduction

The development of agriculture and horticulture in Australia was dependant on the introduction and cultivation of the domestic crop plants of Europe. These were the crops the Europeans were used to growing and their palate preferences were for the tastes of the products of these crops. The introduction of plant species in Australia with European settlement was both deliberate and accidental. Agricultural and horticultural crop plants were brought here by deliberate introduction because generally they can survive only under established cultural practices. However, most of the present pasture grasses and legumes came by way of accidental introduction in the bedding of animals, in ballast and packaging and most likely as seed contaminants in seed samples of introduced crop species.

The problems facing the adaptation of the introduced agricultural and horticultural crop species to the new environments of Australia were many and difficult. The first introduced cereal species wheat, oats and barley were European types and, being adapted to the long photoperiods of the European growing season, were ill-adapted to the shorter days of the Australian growing season. This rendered them too late in ripening in Australia with the consequence that, first, they often suffered from severe drought and produced either no grain at all or very low yields of grain, and second, their late ripening made them very vulnerable to disease attack, especially the leaf and stem rusts of wheat and oats.

Rather fortuitously, the early cereals were most likely mixtures of different types, quite different from the strict pure line varieties now used in crop production. They afforded scope for selection of the early-maturing and disease-resistant types whose superior yielding ability would have been obvious in the late-maturing and disease-ridden mixtures from which they were selected. Thus the first phase of improvement in the temperate cereals was by selection, usually through the eye of the observant farmer.

The next phase of improvement was breeding and selection. In wheat, this was done by such notable breeders as William Farrer at Lambrigg in New South Wales and Hugh Pye at Dookie in Victoria. They were concerned with hybridisation and selection for earliness, disease resistance and improved baking quality. This work, which was

commenced in the 1880s, laid the foundation for the development of the Australian wheat industry. The wheat variety Federation, bred by William Farrer and released for commercial use in 1900, combined the earliness of an Indian wheat with the productiveness and baking quality of a North American wheat. Because of its earliness, Federation could grow in drier, shorter growing season environments than those of the coastal areas where the first agricultural developments took place. Wheats of this type enabled the wheat industry to expand to inland areas of the continent with much lower annual rainfalls.

Although the cereals wheat, barley and oats were the first crops to be successfully adapted through breeding and selection to the agricultural environments of Australia, a range of other crops were introduced early in Australia's agricultural development. These included oilseeds, pulses, summer-grown cereals such as maize and rice, and fibre crops such as cotton and flax.

Crop Breeding in Australia

Crop breeding in Australia is carried out mainly by state government organisations and universities (Downes, 1990; Lazenby, 1986). A number of Australian universities conduct crop breeding programs and are also involved in research training in this area. The CSIRO has a limited involvement in the breeding of temperate and tropical crops in Australia and is more concerned with basic research on the physiology, biochemistry, genetics, tissue culture and molecular biology of crop plants relating to crop yield, product quality and pest resistance. This information is used in improving the efficiency of breeding and selection for crop improvement. Some private companies are also involved in field crop improvement in Australia.

The funding of plant breeding in Australia is in a state of flux at the time of writing. There are moves towards increased privatisation and reduced government spending. This may lead to fewer, larger breeding programs that target a greater range of environments. The benefits of plant breeding to Australia are extremely large but they are probably poorly appreciated by farmers, let alone the general public.

Wheat Wheat is Australia's most important agricultural crop in terms of its volume of production and export. Australian wheat is predominantly bread wheat (*Triticum aestivum*), the cultivated hexaploid species but a small amount of tetraploid or durum wheat (*T. turgidum*) is grown for pasta and noodle manufacture. Wheat varieties, like those of the other temperate cereals (barley, oats, triticale and cereal rye) are adapted to areas of cool growing conditions and moderate to low rainfall in Australia. They are sown in late autumn or early winter. Most of their growth is produced in the spring and they ripen in early summer.

The main problems facing the breeder for improve adaptation of wheat in Australia are increased yield, disease resistance and quality. Breeding for yield involves a number of different approaches. The main activity is concerned with hybridisation and selection within crosses between varieties and parents possessing either high levels of yield or a superior expression of one or a number of its components. Australian wheats are semi-dwarf in habit which ensures more consistent yield levels by reducing lodging under conditions of high fertility, and also appears to bring with it increased yield

potential in the form mainly of increased grain number per spikelet. Another approach to breeding for yield is to increase resistance or tolerance to environmental stresses such as frost and drought. Sprouting resistance, resistance to boron toxicity, and tolerance of acid soils are also breeding aims of various wheat breeding programs.

The most important diseases of wheat in Australia for which breeding for resistance is undertaken are stem rust (*Puccinia graminis tritici*), stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia rubigo-vera tritici*), loose smut (*Ustilago nuda*) and flag smut (*Urocystis tritici*). Breeding work is also conducted on resistance to cereal cyst nematode (*Heterodera avenae*), resistance to leaf blotch (*Septoria tritici*), eyespot lodging (*Cercospora herpotrichoides*), yellow leaf spot, crown rot and barley yellow dwarf virus.

A further important wheat breeding activity in Australia is that of improving milling and baking quality. Important components of milling quality are a high percentage of flour extraction from the grain and an appropriate flour texture. Flour texture can range from hard or vitreous to soft or starchy endosperm types. Both components are strongly influenced by growing conditions but are also of moderate heritability and can thus be modified somewhat by breeding and selection.

Baking quality is influenced by flour texture and both the quantity and quality (e.g. extensibility, dough resilience) of protein in the flour. Most Australian wheat soils are low in available nitrogen so wheat grain produced from such soils has low protein content. This in turn limits the baking quality of flour produced from such wheat. Breeding programs for baking quality improvement aim to produce wheats with improved protein content and quality. Protein quantity, however, is very strongly influenced by the environment and it is difficult to genetically improve its level by any substantial amount.

More recently, a number of significant new markets have been established for Australian wheat including noodle manufacture, biscuits, steam breads (for Asia) and flat breads (for the Middle East). These end-uses impose different quality requirements to those for traditional bread manufacture and are now catered for in Australian breeding programs. A small number of F₁ hybrid wheat varieties have been bred by a commercial company based at Tamworth, New South Wales.

Barley Barley (Hordeum vulgare) is the second most important temperate cereal in Australia and is adapted to a similar range of environments as wheat. It is grown as two different forms, two-rowed and six-rowed which have two or six rows of seeds in the ear. The two types are completely cross-fertile and have similar ranges of variation for most characters. The two-rowed form is grown as a malting barley for the brewing industry. If the grain does not achieve malting quality it is sold for stock feed.

The main breeding aims for increased adaptation of barley in Australia are improved grain yields and malting quality. Important criteria in selecting for increased malting quality include plump grain, high enzyme activity for starch breakdown, high malt extract and low protein. Precautions have to be taken with appropriate seed treatment against the diseases covered smut (*Ustilago hordei*) and loose smut (*Ustilago nuda*).

Net blotch (*Pyrenophora teres*), scald (*Rhynchosporium secalis*), leaf spot (*Drechslera verticillata*) and stem rust are problems in various parts of Australia and are receiving more attention in the quest for a disease-resistant crop. Lodging resistance and the production of winter-type barleys for both grazing and grain are additional breeding aims.

Oats Oats (*Avena sativa*) have a very much smaller volume of production than wheat and considerably less than barley. Oat varieties can be divided into two groups – for grain only, or dual-purpose (grazing & grain), the latter producing a large amount of early vegetative growth which renders them useful for grazing in the late autumn and early winter. The main problems of improving adaptation of oats in Australia relate to increased grain yields in both types, increased vegetative production in the grazing types, disease resistance particularly to stem rust (*Puccinia graminis avenae*), leaf or crown rust (*Puccinia coronata*) and barley yellow dwarf virus, and CCN resistance.

Triticale Triticale is an artificial hybrid cereal species between wheat and cereal rye (see above). There are two forms, hexaploid and octoploid. Hexaploid triticale appears to have the much higher yield potential, and current breeding efforts both in Australia and worldwide are essentially concentrated on this form. There are two general areas of interest in triticale breeding – grain types and grazing/grain types. In terms of grain yield the potential of triticale appears, in some situations, to be at least equal to, if not greater than, wheat. Triticale breeding in Australia is limited because there is limited demand for its grain as an animal feedstuff. However, breeding is in progress for increased grain yield and the incorporation of the semi-dwarf habit for Australian conditions.

Maize In Australia, maize (*Zea mays*) is the major tropical, summer-grown cereal, but is a crop of minor economic importance compared with the winter cereals. Its grain is grown for stock feed and for the production of starch, cornflour and breakfast foods. Maize is also sometimes grown as a green fodder crop. Two other types of maize, sweet corn (*Zea mays saccharata*) and popcorn (*Zea mays everta*), are also grown. Maize breeding in Australia is concerned with selection for yield, appropriate maturity time, drought resistance or tolerance and disease resistance. The main diseases in Australia are leaf blight, head smut (*Sphacelotheca reiliana*) and maize dwarf mosaic. The leaf blights are of two types caused by the fungus *Drechslera turuca* (common leaf blight) and *D. amydis maydis* (leaf blight).

Rice Rice breeding in Australia is aimed at producing high-yielding types of semi-dwarf plant stature, with medium to long grain, early maturity and brown leaf hopper resistance. There is a need for a very early maturing variety with a high degree of cold-hardiness. There is increased effort to produce long-grain and fragrant types to compete with imported rice from Thailand and Pakistan and to satisfy niche markets offshore.

Sorghum The main problems in Australia in breeding for increased adaptation in grain sorghum (*Sorghum bicolor*) are greater resistance to lodging, sorghum midge resistance, appropriate maturity (which is very dependant on temperature of the environment) and disease resistance. The main diseases of sorghum in Australia are

the two fungal diseases, head smut (*Sphacelotheca sorghi*) and rust (*Puccinia purpurea*), and sugar cane mosaic virus (Johnson grass strain). Breeding for increased yield is also undertaken using both selection of open-pollinated populations and commercial (F₁) hybrids.

Lupins Lupins have a whole-seed protein content of 30 to 40% and are therefore a valuable source of stock feed. Modern lupin cultivars are also quite suitable for human consumption but since they are not traditional pulses (except *Lupinus albus*), food use is limited. Lupins were introduced to Western Australia (WA) soon after agricultural development and two species became naturalised over wide agricultural areas of the state.

The main cultivated species in Australia are narrow-leafed lupin (*L. angustifolius*), yellow lupin (*L. luteus*), and white lupin (*L. albus*). The sand plain lupin (*Lupinus cosentinii*) is used in WA as a grazing/forage species. The national lupin breeding program has produced a number of varieties with desirable attributes: low-alkaloid and high-protein content of the seed; resistance to the fungus *Phomopsis leptostromiformis* which grows on lupin stubble over summer and causes lupinosis poisoning in sheep; and resistance to brown leaf spot, Pleiochaeta root rot, and anthracnose (*Collototrichum gloeosporioides*), cucumber mosaic virus seed transmission, and bean yellow mosaic virus.

Field peas Field peas (*Pisum sativum*) have approximately 25% protein in the grain which is used for both human and animal consumption. Field peas are ideal for shorter, temperate growing seasons where sowing is not possible until June (too late for other pulses such as lupins). Early-flowering, erect pea types are being bred to meet a number of markets based on seed type (dun, blue, white). A need also exists for breeding and selection for resistance to the common pests of the crop, particularly the fungal diseases black spot (*Ascochyta pisi*), mildew, and the insects pea weevil (*Bruchus pisorum*).

Chickpeas Attention has been given to selecting for increased adaptation, within a wide range of chickpea (*Cicer arietinum*) types, to the growing conditions of the southern Australian wheat belt. Chickpeas are attractive because of relatively high prices for human consumption. *Ascochyta* blight resistance is a major breeding aim along with seed quality and cooking time.

Canola Canola is the primary winter-growing oilseed in Australia. Oilseeds provide an important human foodstuff and, in particular, fatty acids from which foods such as margarine and cooking oil are manufactured. The oils of certain oilseed crops can also be used in the manufacture of paints, varnishes and resins. The seed residue, after the extraction of the oil, is useful as an animal feedstuff which has high protein content. Commercial canola varieties grown in Australia belong to the species *Brassica napus*. The main breeding aims in canola are for increased yield, reduced pod shattering, disease resistance, especially to the fungal disease blackleg (*Leptosphaeria maculans*), increased oil and protein quantity, and increased oil quality. The desirable oil type for canola quality is low to zero levels of erucic acid and glucosinolates, low levels of

linolenic and high levels of linoleic acid. Indian mustard (*Brassica juncea*) is also bred in Australia for its high oil quality.

Linseed Linseed (*Linum usitatissimum*), which is a different form of the same species as flax, was once grown much more widely in Australia than at present, the oil being used in the manufacture of paints, varnishes and resins. Its decline in production is due to the competition from petroleum-derived oils for the manufacture of these products. Linseed breeding objectives have been to increase seed and oil yield and to incorporate resistance to rust (*Melampsora lini*) and to the disease pasmo. Research by CSIRO Canberra has produced a linseed, known as linola, with an edible oil, low in erucic acid, although market acceptance has been difficult to achieve.

Safflower The seed oil of safflower (*Carthamus tinctorius*) can be used in paints, varnishes and resins. The oil is also of high quality for cooking and can be used in the manufacture of margarine and mayonnaise. Breeding objectives are concerned with increased adaptability and yield and resistance to three main fungal diseases – head rot (*Botrytis cinerea*), root rot (*Phytophthora drechsleri*) and leaf rust (*Puccinia carthami*).

Sunflower The most important summer-growing oilseed crop in Australia is sunflower (*Helianthus annuus*). Most is crushed for the extraction of the edible oil, which has a high content of polyunsaturated fatty acids. The seed residue contains approximately 30-35% protein and is used in stock feed. A large proportion of the sunflower crop in Australia is now sown as F₁ hybrids, with a declining percentage sown to open-pollinated varieties. The F₁ hybrids are generally more uniform in height and maturity than the open-pollinated types. Breeding objectives are for the production of types with increased seed and oil yield, better adaptation to high-temperature and moisture stress, and resistance to the diseases rust (*Puccinia helianthi*), *Alternaria*, sclerotinia stem rot and wilt (*Sclerotinia sclerotiorum*) and charcoal rot (*Macrophomina phaseoli*).

Soybeans Soybean (*Glycine max*) grain is high in protein and oil and thus can be used directly as a human foodstuff or crushed for the extraction of edible oils, the residue constituting a valuable stockfeed. The main breeding aims for soybean improvement in Australia are for varieties for full-season culture in the areas of its production with lodging resistance and resistance to the diseases bacterial blight and bacterial pustule, soybean rust (*Phakopsora pachyrhizi*), *Phytophthora* root rot, and stem rot. Both dryland and irrigated varieties are bred. Coastal varieties require resistance to manganese toxicity.

Cotton Since the 1970s, cotton (*Gossypium hirsutum*) has become a major crop in northern New South Wales and central and southern Queensland. A large and highly successful breeding effort has concentrated on increasing yield, insect resistance (by the exploitation of morphological traits such as the dissected okra-type leaf), quality (fibre strength, length and fineness), adaptation to marginal environments (cooler, shorter seasons), resistance to bacterial blight (*Xanthomonas campestris* var. *malvacearum*) and increased tolerance to wilt (*Verticillium dahliae*). Although most of the breeding effort is directed towards irrigated varieties, dryland cotton is often

grown on a large area when season conditions permit. The best irrigated varieties are usually also superior in a dryland situation.

Sugar Cane The breeding effort in sugar cane has concentrated on increasing yield and ratooning ability. Sugar cane is clonally propagated, consequently some effort has been directed at enhancing the germplasm base and improving crossing methods so as to provide a greater range of material for selection.

Other Crops The following field crops are the subject of breeding programs in Australia: navy beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*), peanut (*Arachis hypogaea*), faba beans (*vicia faba*), various grasses (native and exotic), subterranean clover (*Trifolium subterraneum*), *Stylosanthes* species, lucerne (*Medicago sativa*), annual medics (*Medicago* species), *Leucaena leucocephala* (browse shrub), potatoes (*Solanum tuberosum*), rye (*Secale cereale*), tobacco (*Nicotiana tabacum*), and millet (*Pennisetum americanum*)

PRINCIPLES

- Plant breeding is applied genetics. It involves the generation of new, improved gene combinations (genotypes). The new genotypes are released as varieties and sold to farmers for commercial production.
- Modern breeding is an extension of the plant domestication and selection carried out by ancient farmers, mainly in the centres of origin of the species concerned.
- Plant breeders primarily aim to increase yield per unit area, along with improved resistance to pests and diseases, quality of product, and adaptation to the available environments.
- Plant breeding has four phases: identification of needs and available germplasm; production of new genotypes through crossing (artificial hybridisation); selection to isolate the best new combination; finally testing and evaluation of performance, and variety release.
- Each year a typical breeding program might make 200 crosses, each of which produces several thousand new genotypes, but only one of which is worthy of variety release more than ten years later.
- Naturally-occurring variation in the plant species, and in its close wild and weedy relatives, is the basic raw material for the vast majority of plant breeding activities. Conservation of existing genetic resources is, therefore, vital for future progress.
- When natural genetic variation is lacking, other techniques can be used to try and produce novel variation.
- DNA markers, especially those based on the PCR-reaction, are a powerful tool in plant breeding, and their use will become very common over the next decade. Genomics will increase the pace of progress.
- Biotechnology leading to transgenic (or genetically-modified) plants may be exploited when existing variation or existing techniques are insufficient. The Public's acceptance of transgenic crops is still uncertain. Many ethical, ecological and sociological questions remain to be answered. Transgenic varieties cannot be delivered to farmers without the collaboration of a plant breeding program.

- Some characters manipulated by plant breeders are controlled by single, major genes and follow the simple rules of Mendelian inheritance.
- Many other important characters are controlled by multiple genes of minor but cumulative effect, so-called Quantitative Trait Loci (QTLs). Quantitative genetics is used to analyse these characters and to predict likely progress under selection. DNA markers are used to dissect these characters and to assist in their successful manipulation by breeders.
- The heritability of a character describes the proportion of the total observed variation that is due to genetic, rather than environmental, causes. A character with high heritability is easier to breed. Genotype-by-environment interaction is a constant frustration to progress.
- The breeding methodology employed depends primarily on how the plant reproduces: asexually, sexually by self-pollination (inbreeding), or sexually by cross-pollination (outbreeding).
- In self-pollinating species (most crops), varieties consist of genetically-fixed (homozygous), identical individuals that breed true. Low levels of cross-pollination occur in most self-pollinating species.
- In cross-pollinated plants, varieties consist of populations of heterozygous individuals.
- F₁ hybrid varieties are a special case where every plant is genetically identical but heterozygosity is maximised. Hybrids are produced by manipulation of the system of reproduction during the breeding process. Male-sterility is the most common method.
- Plant varieties are usually registered under federal Plant Breeders' Rights legislation which is a kind of copyright protection. Farmers may retain seed of a PBR-protected variety for their own use but seed cannot be bartered or sold except with the permission of the licence holder. Some varieties are distributed only under individual contracts with farmers.
- The aims of a plant breeding program will usually be regionally-specific since the pests, diseases, climatic, and edaphic characteristics in a particular area will be different from other areas.
- All of the major crop plants grown commercially in Australia have plant breeding programs. The larger crops, such as wheat, are serviced by nationally-coordinated programs with regional centres. The funding is primarily from Government but with substantial input from growers through levies.

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