Plant Breeding and Crop Improvement

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Introductory article

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To respond to the increasing need to feed the world's population as well as an ever greater demand for a balanced and healthy diet there is a continuing need to produce improved new cultivars or varieties of plants, particularly crop plants. The strategies used to produce these are increasingly based on our knowledge of relevant science, particularly genetics, but involves a multidisciplinary understanding that optimizes the approaches taken.

Introduction

In plant breeding the aim is to produce new, improved varieties/cultivars and so we need, as a first requirement of any breeding programme, to release or produce genetic variation in the characters (or traits) in which we are interested. Once such variation is released it is necessary to identify and then select the desired types – those that have a better expression of a particular character or combination of characters. Once identified the selected types need to be stabilized and multiplied for use and exploitation (**Figure 1**).

Written in these terms it appears a relatively simple process, and in many ways the philosophy underlying crop improvement is simple. Although the practical reality is more complex it is possible to identify these three parts and see a framework in which to understand what is being done and what alternatives might exist. Each of these elements is tailored to be appropriate to the particular type of crop, or species, or even the likes and requirements of an individual breeder.

Classical Breeding

Release/production of variation

Conventionally this is achieved through sexual crossing, particularly of cultivated lines, in other words following Mendel's principles. The parents who have expression of the desirable characters between them are intercrossed and the subsequent generations examined for plants with the desired characters in new combinations, i.e. we look for recombinants. This process therefore basically relies on the segregation of alleles at all the relevant genetic loci, during the normal process of meiosis (the reduction divisions that are undertaken to form the egg and pollen cells that fuse at fertilization). At fertilization there is a random fusion of gametes (pollen from the one plant and egg from the other) to give the embryo which develops into the seed. So by the natural process of sexual reproduction, but between plants that the breeder has deliberately chosen, we get offspring that contain novel combinations of the alleles that were originally dispersed between the two parents. Clearly the choice of parents is critical.

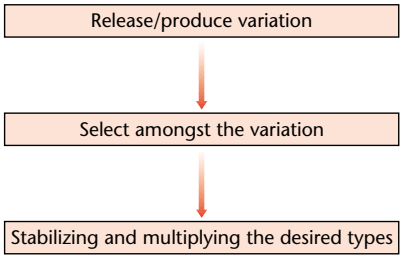

Figure 1 Diagrammatic representation of the major steps in any plant breeding programme.

Sources of variation

The breeder generally uses the natural variation that already exists within the species. For virtually all characters we only need to look or measure any character to observe variation in their expression, and often this reflects not just variation produced by differences in the environment in which the plant happens to be growing, but also genetic variation – variation that is heritable. This naturally occurring source of heritable variation accounts for most of the responses that have been made in plant breeding. However, reliance on this one source of variation does limit the potential for long-term progress, particularly in relation to improving specific characters. So the use of intraspecific variation of existing crop cultivars is supplemented by one of the following:

- Wild, ancestral relatives of the crop itself: these may or may not still be able to cross sexually with the crop species and may be indigenous in another country.
- Inducing the variation that is required: the genetic variation that we see around us actually comes from the occasional and rare mistakes that occur in the otherwise faithful replication of the DNA in all organisms. These occasional mistakes are called mutations and what we see as variation in any character today is the accumula-

tion of such mistakes over a long period of time. The frequency with which mutations occur can be increased and the subsequent variants exploited.

- Developments in the areas of molecular biology and biotechnology: these have extended the possibilities for introducing additional variation in the breeding process (noted later).

Select amongst the variation

The first difficulty is to decide which characters to select. This may seem straightforward but in practice it means trying to put in order of priority what will be needed in the new cultivar not only in relation to improving characters but also in relation to the ones whose expression is already satisfactory in the parents (as the characters will not normally remain unchanged without positive selection). If this did not present sufficient problems, the breeder is also faced with practical difficulties. First, it is not possible to measure every character that might be relevant because there are simply too many for this to be practical. In addition, some characters take a great deal of time and effort to measure, and so may demand more resources than are available. A major problem in a breeding programme is that there is a need to handle large numbers of different genotypes but only small quantities of planting material of each is available. How does the breeder grow the plants such that they display their characters under conditions that resemble those under which they will actually be grown in agriculture? So it is important for the breeder to check the feasibility and relevance of the characters being measured in the context of the reality of how and where the cultivar will be grown.

Connected with the above is the efficiency with which selection can be practised. We note that what the breeder observes is the phenotype but what he needs to select is the genotype (i.e. the heritable part of the variation that is observed). The relation between phenotype and genotype can be written as:

$$\text{Phenotype} = \text{Genotype} + \text{Environment} + (\text{Genotype} \times \text{Environment})$$

Thus, the less that the environment affects the character, either directly or by interacting with the genotype, the better the indication of the genotype that will be gained by simply observing the phenotype. However, many of the characters of interest do not show variation that is easy to classify into discrete classes – i.e. is not major gene determined, such as Mendel investigated, where phenotype and genotype are closely associated. They do in fact show continuous variation (i.e. many different genotypes, with an even greater subtly different range of phenotypes) and are strongly influenced by the environment in which they are grown. For example, yield is a character of immense interest to any breeder but is controlled by many genes and significantly affected by fertilizer levels, husbandry, weather etc.

Breeding objectives and important traits

The general objectives of virtually all breeders of crop plants are to increase the usable yield, increase its stability, ensure the quality and nutritive value, and produce types that suit the particular growing conditions and farming needs.

1. Usable yield. This means that it is not crude yield that is important but the part that can actually be used, eaten, processed, etc. This therefore brings in factors such as the storage life, waste produced and consumer acceptance. Also it means that the use that the crop will be put to is of major importance, i.e. for direct human consumption, animal foodstuff, processing etc., and this must be considered at the outset of the breeding programme.
2. Stability of yield. The fact that some lines/cultivars/varieties do very well in some years or under some particular conditions may be useful but can lead to disaster when they fail because of changes in the growing conditions, a poor year for rain, no fertilizer available, too wet a period at harvest etc. Thus breeding for resistance/tolerance to all biotic and abiotic stresses is a major aim.
3. Quality of the product. This includes nutritional quality and taste and is related to the awareness of usable yield but is concerned with the nutritive value, calorific value, protein content, fat level, vitamin concentration etc.
4. Environmental impact. Agriculture affects any area where it is practised and so generates considerable debate in many parts of the world – and quite rightly so. There are many aspects to this issue and all affect the plant breeder's aims and objectives. There are therefore much clearer calls for more ecologically sympathetic methods to achieve these aims. The production of varieties with disease and pest resistance is an obvious route to follow. Taking this further, if, for example, low-nitrogen input is required then clearly specific varieties will need to be produced that grow best under these conditions.
5. Better adapted. The need to produce varieties that have been selected to grow under prevailing conditions is clear but easily overlooked. This may be the climate of a particular geographical location, the narrow conditions of a local area, the type of agricultural practices used, the needs of the farmer/village/country etc.
6. Prediction. Every breeder knows that it takes a number of years from starting to breed a cultivar until its release to the grower (often 10 years or more). This means that a breeder requires an ability to forecast the

future, i.e. be a crystal ball gazer. So, for example, a breeder might need to assess/guess:

(i) What will growers be requiring in the future?
(ii) What will happen in terms of the emphasis for growers, e.g. what subsidies will there be and what will the political situation be in the future?
(iii) How will climate change have affected growing patterns?
(iv) How will farming systems have changed?
(v) What will be the spectrum of diseases and pests?
(vi) What will the end-users require in the future?

Stabilizing and multiplying the desired types

One of the most important determinants that introduces differences in the details of this part of the breeding strategy is the natural breeding system of the plant. The main natural breeding systems can roughly be classified into inbreeders, outbreeders (outcrossers) and clonally reproduced (i.e. asexual or vegetative propagation). These three main differences in the natural breeding system lead to what are commonly considered the main categories of classical breeding programmes identified and are briefly reviewed here.

Inbreeders

Examples of crops that are inbreeders are wheat, barley, rice, soybean, peas, tobacco, tomato, millet, lentil, flax and chickpea.

These are species that naturally self-pollinate and in commercial practice are grown as true breeding, homozygous lines. So all the individuals of a particular cultivar are genetically identical.

Each generation is produced by allowing the plants to self-pollinate in each cycle of the breeding programme so that while the trialling and selection process is proceeding the plants are becoming more inbred. When the finished cultivars are selected they will breed true from seed (they are genetically homozygous). So the genotype is now fixed and the cultivar can be multiplied simply by letting it set seed (isolated from any other genotypes of this crop – as some pollen is likely to pass between them).

There are two main methods by which selection is achieved during this inbreeding process: bulk method and pedigree method.

Bulk method

The bulk method (**Figure 2**) starts with the creation of genetic variation by the hybridization between two parents ($P_1 \times P_2$). The F_1 and several subsequent generations, often up to and including the F_5 generations, are grown as bulk populations. No conscious selection is imposed on these generations and it is assumed that the genotypes most suited to the environment in which the bulk populations

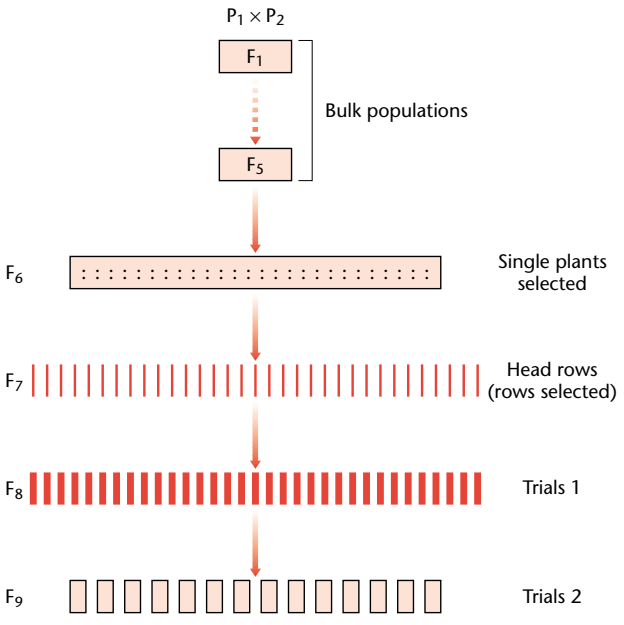

Figure 2 The bulk method.

are grown will leave more offspring and hence predominate in future generations. It is therefore very important that the bulks are grown in an environment that will be similar to that needed for the resulting cultivars. At the next stage, individual plants showing desirable characteristics are selected. From each selected plant, a plant (or head) row is grown and the produce from the best lines/rows are selected, bulk harvested, for initial yield trials, and resown for multiplication.

This method is one of the least expensive methods of producing populations of inbred lines. The disadvantage of this scheme is the length of time from initial crossing until yield trials are grown. In addition, it has often been found that the natural selection that is relied on in the early, bulked generations is not always that which favours characters thought desirable for growth in agricultural practice.

Pedigree method

In a pedigree breeding scheme single plant selection is carried out at the F_2 through to the F_6 generations. Again, the scheme begins by hybridization between chosen homozygous parental lines ($P_1 \times P_2$), and F_2 populations are obtained by selfing the heterozygous F_1 plants. Single plants are selected from amongst the segregating F_2 population. The produce from these selected plants are grown in plant/head rows at the F_3 generation. The most desirable single plants are selected from the 'better' plant rows and these are grown in plant rows again at the F_4 stage. This process is repeated, but with an increasing shift from individual plant to row performance, until plants are near homozygous (e.g. F_5). At this stage the most

productive rows are bulk harvested and used as seed source for initial yield trials in F_6.

In addition to being laborious (as a considerable amount of record keeping is required) and relatively expensive, the reliance on individual plant selection is inefficient and leads to the loss of valuable genotypes before they are fully tested. However, the greater control over the selection and the defined pedigrees make this a preferred method in many crops.

Cross-pollinated (or outbreeding) crops

Some examples of cross-pollinated crops are alfalfa, rye, herbage grasses, forage legumes, red clover, some maizes, perennial ryegrass, sugar beet and oil palm.

The selection of new cultivars of cross-pollinated crop species is a process that changes the gene frequency of desirable alleles within a population of mixed genotypes while trying to retain a high degree of heterozygosity. So it is really the properties of the population that are vital, not individual genotypes (as in self-pollinating crops). Instead of resulting in a cultivar for release that is a uniform genotype, the population will be a complex mixture of genotypes, which together give the desired performance. There are basically two different types of outbreeding cultivars, which are determined by the methods of their maintenance and multiplication: open-pollinating populations and synthetic cultivars.

Open-pollinating population cultivars

In open-pollinating populations, selection of desirable cultivars is usually carried out by mass selection, recurrent phenotypic selection or selection with progeny testing. The maintenance of these cultivars is through open-pollinated populations with uncontrolled (random) mating.

Mass selection

Mass selection is based on the same underlying philosophy and assumptions as the bulk method for inbreeding species. It is a very simple breeding scheme, which uses natural environmental conditions to alter the genotypic frequency of an open-pollinating population. A new population is created by cross-pollinating two different existing open-pollinating populations. In this case a representative set (any single plant will not, of course, be fully representative of the populations) of individuals from each population will be taken to be crossed.

The seed that results from such a set of crosses is grown under field conditions over a number of seasons. It is assumed that crossing will be at random and so result in a population quickly moving towards equilibrium which can be maintained, as a population, for exploitation. It should be noted that care needs to be exercised in isolating this developing population from other crops of this species that might happen to be growing within pollination distance!

Recurrent phenotypic selection

Recurrent phenotypic selection (**Figure 3**) tends to be more effective than mass selection. A population is created by cross-pollination between two (or more) populations to create what is referred to as the base population. A large number of plants are grown from the base population and a subsample of the most desirable phenotypes are identified and harvested as individual plants. These plants are then randomly mated to produce a new improved population. This process is repeated a number of times – it is a recurrent process! What can be exploited as cultivars can be extracted at any stage tested and distributed to growers.

Synthetic cultivars

A synthetic cultivar basically gives rise to the same end result as an open-pollinated cultivar, the main difference being that a synthetic cultivar is continually reproduced from specific parents, whereas if it is left to open-pollinate to produce over generations, it will change its genetic make-up as a population. This means that farmers need to return to the seed companies for new seed when they re-sow the crop.

The breeding method used for the development of synthetic cultivars is dependent on the ability either to develop homozygous lines for use as parents or to be vegetatively propagated so that any genotype can be maintained as a parent.

Developing hybrid cultivars

Examples are Brussels sprouts, kale, maize, onions, rape, sorghum and tomato.

A special case that arose from developing synthetic cultivars is the idea of hybrid cultivars from just two parents. In theory any species might be used in hybrid production but commonly it is outbreeding species that are actually exploited in this way, although maize is exploited in this way and can certainly be inbred.

At the beginning of this century there was a general awareness, especially in the USA, that the means being

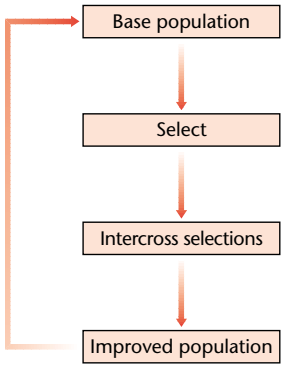

Figure 3 Recurrent phenotypic selection.

used to develop new corn (maize) cultivars (mass selection and ear-row selection) were less effective than had been hoped for in breeding more productive cultivars or increasing yield. Another approach was suggested from the knowledge that hybrids produced by cultivar × cultivar crosses often showed heterosis (i.e. produced yields greater than the better parent). It was then proposed that this could be exploited by manually detasselling one maize line (designated as the female parents – i.e. removing the male flowers) in plots also containing the second line, so that seeds produced on the line designated as female must have been pollinated by the pollen from the flowers of the male line. Thus it was possible to create a population that was entirely comprised of hybrids and to use it for commercial planting. This is known as a single-cross hybrid.

The major steps in producing hybrids are very similar to those for producing a synthetic cultivar, namely:

1. Development of inbred lines to be used as parents.
2. Test cross these lines to identify two that combine to give the best progeny.
3. Guard the inbred pairs that when crossed give the best hybrid cultivars and use them to produce the hybrid cultivar when desired.

There are hardly any agricultural crops where hybrid production has not at least been considered, although hybrids are exploited in relatively few crop species. The reasons behind this are first that not all crops show the same degree of heterosis (superiority over the better parent) found in maize and secondly that it is not possible in many crops to find a commercial seed production system that is economically viable. Indeed if maize had not had separate male and female reproductive organs and hence allowed easy manual detasselling, hybrid cultivar development might never have been developed, or acceptance would have been delayed at least 20 years, until cytoplasmic male sterile systems were available.

Hybrid cultivars have been developed, however, in sorghum, onions and other vegetables using a cytoplasmic male sterile (CMS) seed production system; in sugar beet and some *Brassica* crops (mainly Brussels sprouts, kale and rapeseed) using CMS and self-incompatibility to produce hybrid seed; in tomato and potato using hand emasculation and pollination.

If hybrid cultivars are to be developed from a crop, then the species must:

- show a high degree of heterosis;
- be capable of being handled so as to produce inexpensive hybrid seed;
- not easily be produced uniformly by other means, and have a high premium for crop uniformity.

Heterosis

The performance of a hybrid is a function of the genes it receives from both its parents but can be judged by its phenotypic performance in terms of the amount of heterosis it expresses. Many breeders (and geneticists) believe that the magnitude of heterosis is directly related to the degree of genetic diversity between the two parents. In other words, it is assumed that the more the parents are genetically different the greater the heterosis will be. To this end, it is common in most hybrid breeding programmes to maintain two, or more, distinct germplasm sources (heterotic groups). Breeding and development is carried out within each source and the different genetic sources are only combined in the actual production of new hybrid cultivars. For example, maize breeders in the USA observed significant heterosis by crossing Iowa Stiff Stalk breeding lines with Lancaster germplasm. Since this discovery these two different germplasm sources (heterotic groups) have not been intercrossed to develop new parental lines but, rather, have been kept genetically separated.

Development of clonal cultivars

Examples are bananas, cassava, citrus, potatoes, rubber trees, soft fruit (raspberry, blackberry, strawberry), sugarcane, sweet potatoes and top fruit (apples, pears, plums, etc).

Clonal crops are basically perennial, although several crop species, particularly those where the actual unit of clonal reproduction is the part of the plant that is exploited (e.g. tubers of potato and sweet potato), are treated in agriculture as annual crops and replanted in each crop cycle. Clonal crops also include many long-lived tree crops (e.g. apple, cherry, rubber and mango) which can be productive crops for many decades after being established.

Methods of propagation are various. Rosaceous top fruits, citrus, avocado and grape involve budding and grafting onto various rootstocks. Leafy cuttings are used for pineapple, sweet potato and strawberry. Leafless stem cuttings are used in sugarcane and lateral shoots are used for banana and palms. There is also, for a number of species, the potential for clonal reproduction via tubers (swollen stems), e.g. potatoes.

In general, clonal crop species are often outbreeders that are basically intolerant to inbreeding. Individual clones are genetically heterozygous and so it is easy to exploit the presence of any heterosis. If maize could be easily reproduced asexually there would be little or no need to develop hybrid corn cultivars because the highly heterozygous nature of a hybrid line could be fixed by vegetative reproduction.

The process of developing a clonal cultivar is, in principle, very simple. Breeders generate segregating progenies of seedlings, select the most productive genotypic combination and simply multiply this asexually; thus

there is no need for extra procedures to stabilize the genetic make-up (i.e. it relies on asexual reproduction, thus avoiding problems relating to genetic segregation arising from meiosis). Despite the apparent simplicity of clonal breeding it should be noted that while clonal breeders have shared in some outstanding successes, it has rarely been due to such a simple process.

In the case of potato, the length of the process is, in part, related to a slow multiplication rate, around 1:10 per generation. In addition, seed tubers are bulky and require large amounts of storage space. To accommodate planting material for one hectare of potatoes will require 2241 kg of seed tubers. With many other clonal species the time from crossing to cultivar release can be a very lengthy process. In apple breeding, for example, it is often said that if a breeder is successful with the very first parent cross combination, then it is still unlikely that a cultivar will be released (from that cross) by the time the breeder retires! In this case there is the obvious difficulty in the time taken from planting an apple seed to the time that fruit can be evaluated.

New Genetic Approaches

Tissue culture (*in vitro*) techniques

A variety of techniques (micropropagation, haploid production, protoplasts, embryo culture, apical culture, somatic embryogenesis, etc.) have been developed under the title of tissue culture and so just two particular examples are noted here to give an idea of the possible applications.

Haploidy

Establishing true breeding, homozygous, lines is an essential part of developing new cultivars in many crop species. These homozygous lines are used either as cultivars in their own right (i.e. for inbreeding crop species) or as parents in hybrid variety development. Traditionally, plant breeders have used the process of selfing or mating between close relatives to achieve homozygosity, a process that is time-consuming. Therefore the opportunity to produce plants from gametic, haploid cells has been the goal of many plant breeders as this technique would produce instant inbred lines once the chromosomes of the haploids are doubled.

The genetic phenomenon critical to obtaining homozygous lines is the formation of haploid gametes by meiosis. During this type of cell division, the chromosome number is halved and each chromosome is represented only once in each cell (assuming the species is basically a diploid one). If such gametic, haploid cells can be induced to develop into plantlets (i.e. we encourage the development of the sporophyte – note: lower plants often have this as a specific phase of the life cycle) a haploid plant can develop which can then be treated to encourage its chromosomes to double, to produce a completely homozygous line (a doubled haploid).

Techniques used for producing haploids *in vitro*

Although haploidy is a very attractive technique to many plant breeders the natural occurrence of haploid plants is rare. However, the use of plant tissue culture has allowed the production of plants from gametic cells cultured *in vitro* at a higher frequency.

Although haploid plants can be regenerated from both male and female sex cells, it is generally the male cells (microspores or pollen) that have proven most successful in the regeneration of large numbers of haploid and doubled-haploid lines. This is partly because of the ease with which pollen, as opposed to eggs, can be collected, and partly because many more pollen grains than eggs are produced.

There are a number of methods of haploid induction that are not directly related to tissue culture but the most widely applicable are via the culture of anther or microspore (immature pollen grains) *in vitro*.

In vitro multiplication

In vitro multiplication of breeding lines can have two main benefits (particularly in clonal species) in relation to plant breeding programmes.

1. Plants propagated *in vitro* can generally be initiated and maintained in a disease-free state, and so can be used to help maintain stocks of breeding lines; facilitate long-term germplasm storage; and facilitate international exchange of material.
2. Short generation times and fast growth means that rapid increases in plant number can be readily achieved.

Both the above have particular importance to clonal crops, because these tend to have a relatively low multiplication rate as a result of their vegetative mode of propagation and are particularly susceptible to viral and bacterial diseases, which tend to be multiplied and transmitted through each clonal generation.

Good examples of maintaining a disease-free status and offering rapid plant regeneration potential include potato and strawberry. Other, perhaps less well-developed examples, include *in vitro* propagation of date and oil palms. In these crops it was found that more rapid plant regeneration would indeed offer an alternative to the slow and lengthy process of propagating side shoots in date palm and a more uniform planting material in the case of oil palm. However, in date palm the process is still very genotype dependent, and with oil palm there initially proved to be an unacceptably high frequency of sterile palms produced.

Plant transformation

The stable introduction of specific genes into plants represents one of the most significant developments affecting the production of crop species in a continuum of advances in agricultural technology. The progress in this area has depended largely on the tissue culture systems having been developed which, at least, initially, provide an amenable vehicle for the transformation induction.

The term transformation comes from that used for a much longer period, bacterial transformation, in which DNA has been successfully transferred from one isolate to another or another species of bacteria, and integrated into the genome. It was shown that the stably transformed bacteria then expressed the new genes and displayed appropriately altered phenotypes. In eukaryotes, transformation has a further complicating dimension, at least in many plants' breeding contexts. The transforming DNA must not only be integrated into a chromosome, it must be a chromosome of a cell, or cells, that will develop into the germline. Otherwise the transformation will not be passed on to the next generation.

Using plant transformation techniques it is possible to transfer single genes (i.e. simply inherited traits) into plants, to have such transgenes expressed and for them to function successfully. Theoretically at least, specific genes can be transformed from any source into developed cultivars or advanced breeding lines in a single step. Plant transformation, therefore, would appear to allow plant breeders to bypass barriers that limit sexual gene transfer and to exchange genes (and traits) from unrelated species between which sexual hybridization is not possible. These recombinant DNA techniques, apparently, allow breeders to transfer genes between completely unrelated organisms. For example, bacterial genes can be transferred and expressed in plants. This appears to break the barrier that sexual reproduction generally imposes. However, as we learn more about the DNA, and hence the genes involved, the perspective of the picture changes somewhat, with increasing direct evidence of the presence in different species of the same basic gene, or clear variants of it, and demonstrations of the greater conservation of genetic material (synteny) during evolution than we expected. Also, we are being reminded of the existence of parallel natural processes for much of what we regard as novel. For example, bacteria, viruses and phages already have successfully evolved mechanisms to transfer genes just in the way we regard as being so alien! But clearly, the new techniques are allowing modern plant breeders to create new variability beyond that existing in the currently available germplasm on a different scale and in a different time frame from that which was previously possible.

Although plant transformation has added (some say dramatically) to the tools available to the breeder for genetic manipulation, it does have limitations. Some of the limitations will reduce with increased development of methodologies, others are inherent to the basic approach. At present recombinant DNA techniques can generally only transfer rather limited lengths of DNA and so tend to be restricted to the transfer of single genes. This means that they are very effective where the trait can be substantially affected by a, or a few, gene(s) of large effect. Another restriction that is imposed currently is that the techniques are only readily applied to genes that have been identified and cloned. The number of such desirable genes is still modest, but increasing rapidly.

Some applications of genetic engineering to plant breeding

Already there is a growing list of crop species that have proved successful hosts for transformation including alfalfa, apple, carrot, cauliflower, celery, cotton, cucumber, flax, horseradish, lettuce, maize, potato, rapeseed, rice, rye, sugarbeet, soybean, sunflower, tomato, tobacco and walnut.

Initial cultivar development using recombinant DNA techniques has focused on modifying or enhancing traits that relate directly to the traditional role of farming. These have included the control of insects, weeds and plant diseases. The first genetically engineered crops have now been released into large-scale agriculture (including maize, tomato, canola, squash, potato, soybean and cotton) and other species are already in the pipeline. More recently work has focused on altering end-use quality (including oil composition, starch, vitamin level and even vaccines).

Cautions and related issues

There have been a number of concerns that have arisen over the past few years as the application of plant transformation technology has expanded and particularly as new transgenic crops have been released into commercial cultivation. Plant breeders need to be aware of the concerns as well as the regulations that apply to plants derived using recombinant DNA. As well as the general social and environmental concerns the breeder must check that the techniques being used are the most effective for what is to be achieved and not simply assume that high tech means most efficient!

Molecular markers in plant breeding

Although plant breeders have practised their art for many centuries, genetics is a subject that really only came of age in the twentieth century with the rediscovery of Mendel's work. Since then research in genetics has covered many aspects of the inheritance of qualitative and quantitative

traits, but plant breeders usually still have little, or no, information about:

- the locations of many of these loci in the genome or on which chromosome they reside;
- the number of loci involved in any trait;
- the relative size of the contribution of individual alleles at each loci on the observed phenotype, except where there is an obvious major effect (e.g. height and dwarfing genes).

The idea of using markers

The idea of associating easily visualized markers in plants with loci affecting qualitative and quantitative variation in traits of interest to plant breeders is not new, and was first proposed by Sax in 1923. The basic idea is relatively simple. If a trait or characteristic is difficult to score (e.g. it shows continuous variation; assessment is detailed and time consuming; or the trait is only expressed after several years of growth), an easily scored marker that was determined by a locus closely associated with that affecting the character would be an attractive alternative way to monitor the locus of interest.

The characteristics of a good marker system include the following:

1. The markers are easy, quick, and inexpensive to score.
2. The markers themselves have no deleterious effects on fitness and no effects on other traits, including undesirable epistatic interactions with any other traits.
3. There is a high level of variation exhibited.
4. They are stable in expression over environments.
5. Assessment can be made early in the development of the plant (seedling level), and/or in tissue culture.
6. The scoring should be nondestructive in terms of the whole plant, so that desirable individuals can be selected and still grown to maturity.
7. Codominance in expression of the alternative alleles, so that heterozygotes can be differentiated from either homozygous dominant genotype.

Types of marker systems

The types of markers that can and have been used in plant breeding include:

1. Morphological markers – which are basically those that you see by simply looking at a plant's phenotype, including characters such as pigmentation, dwarfism, leaf shape, absence of petals, etc.
2. Biochemical markers – such as isozyme markers. Isozymes (isoenzyme) are variant forms of an enzyme, which are functionally identical but can be distinguished by electrophoresis – i.e. when placed in an electric field. Under these circumstances the different forms of the enzyme will migrate to different points in the electric field depending on their charge, size and shape.
3. Molecular markers – these represent the variation that is present and can be detected at the level of DNA. There are basically two systems (PCR and non-PCR based) by which molecular markers are generated and their distinction need not detain us, but it is worth pointing out that molecular markers are simply differences in the DNA between individuals, groups, species taxa etc. Clearly the type and level of variation in DNA that we would want to examine is different depending on what level of distinction we are interested in and what questions we are answering. But the main characteristics of molecular markers are that: they are a ubiquitous form of variation; they are free from environmental influence; they show high levels of polymorphism; they have no discernible effects on the phenotype; and they can be detected using only small pieces of tissue.

Given the above characteristics of molecular markers, particularly their relatively unlimited numbers, it is no surprise that the advent of the possibilities of molecular markers in the 1990s was greeted with some excitement and is seen as providing a major change in the potential to exploit the ideas for using markers advocated some 70 years earlier.

Future Potential

Plant breeding will continue to be highly dependent on classical techniques but will undoubtedly increase in efficiency and effectiveness by the addition of these new approaches, which will be used in parallel with the more classical ones. Thus the future will see the range of techniques expanding in such a way as to maximize their benefits by their integrated exploitation.

Further Reading

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