



secure world food supply and sustainable agricultural systems, plant breeders will have to: (1) increase crop yields and product quality still further; (2) develop varieties with wide adaptation as well as varieties for local, specific environments; (3) produce varieties which have improved resistance to various abiotic stresses and which make better use of crop inputs; (4) develop varieties with better resistance to pests and diseases and thereby less dependence on agrochemicals; (5) provide crops and varieties with a wider range of end use. As before increased yield and product quality will be the main breeding objective or to be more precise, production of maximum yield of saleable products at an economical level of input and with a minimum of negative environmental effects. However, most of the other breeding objectives, although often major or dominant features of a breeding programme, indirectly affect yield and often also quality.

To successfully deal with all the above demands in any crop requires access to a wide range of germplasm and a comprehensive and integrated long-term breeding system that makes maximum use of classical breeding methods as well as the new biotechnologies (Bosemark, 1993). However, commercial breeders are always under pressure to produce and market a continuous flow of varieties with improved performance and incorporating new desirable traits. This requires varietal development procedures that result in rapid genetic gain, but which, as a consequence, also lead to rapid restriction of genetic variation and long-term genetic advance. On the contrary, a low or moderate

selection pressure imposed on a broad-base population is unlikely quickly to produce varieties but favours recombination and retention of genetic variation and thereby long-term genetic progress. This fundamental difference between short-term and intermediate to long-term genetic improvement makes it necessary to separate these breeding operations. Figure 1 illustrates such a separation of the operations related to short-term varietal production, intermediate-term population improvement and long-term genetic resources development. The nature and volume of genetic materials employed, the breeding efforts spent, the financial resources allocated and the classical selection methods most commonly employed, are all indicated in the figure. Although such a resource allocation is likely to vary considerably between crops, the principles and the operational structure should be applicable to both self- and cross-pollinated crops and be relevant to all breeding objectives mentioned earlier. In the following I will use this structure to discuss the selection methods best suited to the different operations and how recent developments in cell biology, molecular biology and genetic engineering, when integrated with the classical methods, are likely to impact on practical plant breeding in the years ahead.

### SHORT-TERM VARIETAL PRODUCTION

Short-term breeding operations are designed to quickly develop new competitive varieties and hybrids and usually involve pedigree and backcross selection to produce new lines or

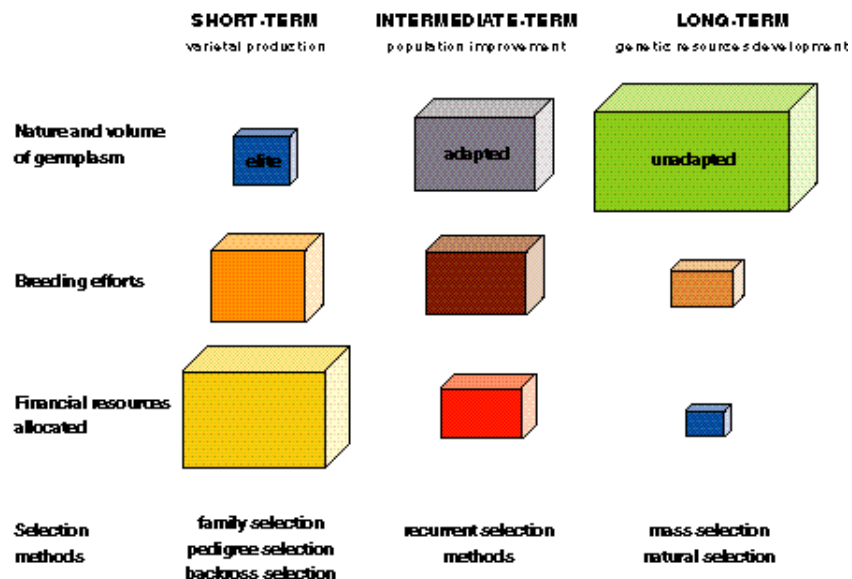


Figure 1.- Resource allocation in plant breeding.

to incorporate new traits into an existing and otherwise useful genotype (Fig. 1). Of particular interest in these contexts is the access to RFLPs and other molecular markers, making possible quick and reliable transfer of genes governing qualitative as well as quantitative traits, both between lines and cultivars and from various genetic resources to cultivars. High density molecular linkage maps now exist for most major crop species (Table 1) and have already been used to identify and facilitate the transfer of genes associated with insect resistance, virus resistance, resistance to fungal pathogens and nematodes as well as tolerance to various abiotic stress conditions. A good example is rice where some 25 genes involved in 10 traits, mainly disease resistances, have now been tagged with RFLP-markers and where IRRI scientists have used markers to transfer and pyramid multiple genes for resistance to bacterial blight into elite lines (Toenniessen, 1993). The fact that all major groups of biotic and abiotic stress factors, to which improved resistance will have to be obtained in the years to come, are represented among the examples is very encouraging. Other breeding operations in which molecular markers are helpful include estimation of genetic diversity of germplasm and finger-printing of varieties and breeding lines. They can also help to dissect complex traits with quantitative inheritance into single gene components, as recently exemplified by the RFLP mapping of genes conferring resistance to rice blast (Wang et al., 1994). Of all developments in molecular biology so far, that of RFLPs and other DNA-markers is likely to be the most universally useful to the breeder. All major plant

breeding organizations have also equipped themselves with the laboratory and computer facilities required for a cost-effective, large-scale implementation of marker-based methodologies in practical breeding. In due course this will permit improved varieties to be produced more quickly than before but also result in a shorter life-span for any given variety.

### INTERMEDIATE-TERM POPULATION IMPROVEMENT

Varietal development in most crops relies to a large extent on the exploitation of the genetic variation among a very limited number of source populations. It has taken several cycles of selection to accumulate in these varieties or lines the many genes for adaptation, quality, pest and disease resistance and yield, required in a modern cultivar. Although this stepwise upgrading of the elite germplasm has been very successful, the high selection pressure has, inevitably, lead to loss of variability and loss of favourable genes linked to unfavourable. The more efficient recycling of recombinant line varieties and elite inbreds now possible with the help of marker assisted backcrossing, is likely to lead to an increased use of this breeding method. However, too strong emphasis on single-cross source populations may result in a dangerous narrowing of the genetic base of the breeding programme and a corresponding widening of the genetic gap between the elite materials and the rest of the gene pool. To counter such a development and to secure continued breeding progress it is important that, parallel with refinements in pedigree and backcross breeding, go

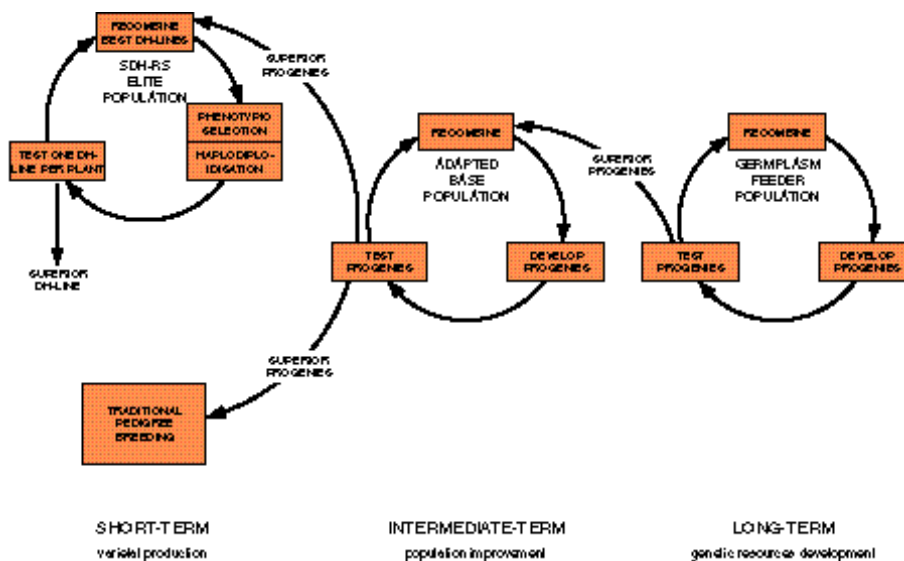


Figure 2.- Outlines of a comprehensive breeding system.



development and continuous improvement of source populations designed to capitalize on a broad, adapted gene pool, as well as the genetic variation available in primitive cultivars and wild relatives of crop species.

Having been originally developed to improve natural and synthetic populations of maize as sources of inbred lines, recurrent selection methods are now widely used in population improvement, not only in crosspollinated species, but also, increasingly, in self-pollinated crops. The composition of these populations and the choice of selection procedure should be based on the kind of gene action governing the trait to be selected for, as well as the type of variety aimed at. Although the principles of recurrent selection are equally valid for autogamous as for allogamous crops, the difficulties in producing the necessary intercrossings in each selection cycle have discouraged its use in self-pollinated crops. However, in self-pollinated species nuclear male sterility may be introduced into a population to facilitate the crossings in the recombination phase, so called male-sterile facilitated recurrent selection. Correspondingly, in cross pollinated species, where self-incompatibility prevents production of selfed seed, the introduction of an  $S^F$  gene and a gene for nuclear male sterility will facilitate both selfing and  $S_1$ -progeny selection and recombination. As has already been indicated, recurrent selection methods are not intended to be used alone but should be integrated with other selection methods used in a comprehensive breeding system. Since all recurrent selection involves testing progenies of selected individuals, superior RS-progenies may enter a pedigree breeding programme aimed at developing recombinant line varieties or inbred lines for hybrid production and thus provide the inflow of new variation into the short-term varietal production programme. In the same way, genetic variation from upgrade genetic resources populations can be fed into the adapted populations. For crops where large-scale haplo-diploidization is possible, Gallais (1988, 1993) has shown that in most situations the best recurrent selection method is recurrent selection based on the phenotype of the doubled haploid with only one line tested per plant. This method, called "single doubled-haploid descent recurrent selection", is also one of the best methods of line development and an interesting alternative or adjunct to pedigree selection in short-term varietal production.

### LONG-TERM GENETIC

## RESOURCES DEVELOPMENT

Access to a wide range of germplasm is essential if we are to deal with both existing problems and emerging problems in pest and disease control, soil problems as well as the postulated impact of atmospheric changes and global warming. Although the volume of material initially sampled or available in gene banks may be very large, in the hands of the private breeder it usually boils down to a few populations, based on crosses between adapted and primitive germplasm. Being a long way from commercial use, such material is likely to receive only a small share of the total breeding effort. Consequently the selection methods need to be cheap and the selection cycles short. In most situations recurrent mass selection and/or half-sib selection should be appropriate. Until improved by several cycles of selection genetic resources populations need to be kept separate. However, once the performance approaches that of the adapted breeding material, germplasm from these populations may be fed into the elite populations.

The time and effort required to develop the kind of genetic resources populations discussed, obviously depends both on the crop and the material that went into the populations. However, experiences with several crops, including maize, sorghum and sugar beet suggest that relatively few cycles of recurrent mass selection may result in populations sufficiently adapted and domesticated to be used as germplasm feeder populations as outlined in Fig. 2. Even so, due to the steadily increasing pressure on commercial breeders to produce new varieties, most of them will be reluctant to engage in this kind of work or will do so in a very limited way. Thus, whenever possible, private breeders should support and collaborate with public breeders and researchers in pre-competitive research and pre-breeding utilizing primitive or wild germplasm

## HYBRID BREEDING AND NOVEL, ENGINEERED MALE STERILITY/RESTORER SYSTEMS

With yield being the most important breeding objective, it is important to make maximum use of hybrid vigour in as many crops as possible. In principle, any crop species showing pronounced inbreeding depression should be a potential candidate for hybrid breeding. So far,

in many crops that meet this requirement, hybrid development is hampered or prevented by lack of a reliable and cost-effective method of pollination control. However, recently, the possibility to introduce male sterility via genetic transformation has opened the possibility for hybrid seed production in a wide range of cross-pollinated crops. The first and best known of these novel male-sterility systems is that developed by Plant Genetic Systems (PGS) in collaboration with University of California (Mariani et al. 1990,1992). This system is now employed in hybrid breeding in oilseed rape conducted by PGS and has been licenced for use in maize, rice and tobacco. At least three other organizations have filed patents on systems of genetically dominant male sterility, and there is reason to believe that more will follow in coming years. This is important, since, besides offering higher yield potential and improved crop uniformity, hybrid breeding makes possible to combine in a single hybrid genes for disease resistance present in the parental inbreds and governing resistance to different diseases or offering protection against different races of the same pathogen.

### THE IMPACT OF BIOTECHNOLOGY AND GENETIC ENGINEERING

Although genetic engineering is unlikely to play a major role in crop improvement until well into the next century, it is important for an assessment of its likely long-term impact to observe that the emphasis of current research and development work, as well as the products of such work already obtained, very largely concern resistance to pests and diseases, environmental stress factors and various quality traits. These traits are precisely those, discussed earlier, which will reduce dependence on agrochemicals, result in increased yields and improved product quality and thus contribute to a more sustainable agriculture. Although, only the Calgene transgenic tomato has as yet reached the market, up to 1992 there had been a total of 675 approved field releases with transgenic plants, representing 31 crop species (Ahl Goy et al., 1994). However, of the 675 releases, tomato, tobacco, oilseed rape and potato account for 428 and cereals, represented by maize and rice, only for 68. This is a reflection of the difficulties encountered when attempting to adapt *Agrobacterium*-mediated transformation systems to cereals and other monocotyle-

Table 1. Major crop species for which molecular linkage maps have been developed.

Sorghum		Soybeans
Wheat	Maize	Millet
Potato	Barley	Sugarbeet
Oat	Rye	Rice
Tomato		Rapessed

donous species, which are normally resistant to *Agrobacterium* infection. However, recently great progress has been made in the development of particle bombardment techniques in which DNA-coated tungsten or gold particles are accelerated into intact cells or organized tissues. Today protocols for particle gun mediated transformation have been developed that permit to engineer all major cereals and a number of

Table 2. Crop plants for which protocols for particle gun mediated transformation have recently been developed.

<b>Dicots</b> Soybean Tobacco Cotton Cranberry	<b>Monocots</b> Rice Wheat Maize Rye Barley Sugarcane Sorghum
<b>Tree species</b> Poplar White-spruce Papaya	



Table 3. Traits and genes for plant genetic engineering.

<ul style="list-style-type: none"> <li>◇ Male sterility/restorer systems</li> <li>◇ Increased starch content</li> <li>◇ Herbicide resistance/tolerance</li> <li>◇ Improved nitrogen use efficiency</li> </ul>
<ul style="list-style-type: none"> <li>◇ Modified starch biosynthesis</li> <li>◇ Modified fatty acid composition</li> <li>◇ Improved storage proteins and aminoacid composition</li> <li>◇ Altered fruit</li> <li>◇ Ripening/storage characteristics</li> <li>◇ Flavour enhancers</li> </ul>
<ul style="list-style-type: none"> <li>◇ Insecticidal Bt endotoxin genes</li> <li>◇ Protease inhibitors</li> <li>◇ Lectin antifedants</li> <li>◇ Chitinase genes</li> </ul>
<ul style="list-style-type: none"> <li>◇ Drought tolerance</li> <li>◇ Metal tolerance</li> <li>◇ Improved nutrient uptake</li> </ul>
<ul style="list-style-type: none"> <li>◇ Viral coat protein genes</li> <li>◇ Chitinase genes</li> <li>◇ Glucanase genes</li> <li>◇ Antibacterial protein genes</li> <li>◇ Antifungal protein genes</li> <li>◇ Enhanced lignin biosynthesis</li> </ul>

other recalcitrant species, often in a genotype-independent fashion (Table 2) (Christou, 1993).

Although the major limitation to the use of genetic engineering in plant breeding still is shortage of useful genes, a look at a far from complete list of target traits and genes currently worked on (Table 3), clearly shows the potential of the technique. With the present emphasis on the isolation of genes using molecular genetic approaches in maize, tomato, wheat, rice and most notably, *Arabidopsis*, the number of genes cloned is likely to increase enormously. Another development of great importance to plant breeding is comparative genome mapping, which implies studying different species for the parallelism in gene order along the chromosomes, as well as breakpoints responsible for differences in gene order. Results of such comparative mapping suggest that remarkably few macroevolutionary events may distinguish plant species. Thus comparative genome mapping in wheat, rye, barley, oats, rice, maize, sorghum and millet has shown that portions of the maps are co-linear, which will enable the reconstruction of an ancestral map of the primeval grass from which all the cereals evolved some 60 million years ago (Moore, 1993). Since comparative genome analysis will provide both an indication of the sources of potential traits and DNA markers to assist in the isolation of the underlying gene, comparative mapping is likely to have a major impact on plant breeding. Although, so far advancement in molecular techniques almost entirely emanate from research in industrialized countries, they may in a longer perspective be of even greater significance to the people in the developing world. Recognizing this, the Rockefeller Foundation has spent more than US\$ 33 million in support of rice biotechnology research.

In spite of the importance of biotechnology and genetic engineering in breeding new crop varieties, the new technologies are not going to take the place of classical plant breeding methods, nor diminish their value. On the contrary, further refinement in these methods and better knowledge of classical genetics are requisites for a rational use of new tools such as molecular markers. It is therefore important, that the less glamorous areas of classical plant breeding research continue to receive support, and that such studies, as far as possible, are closely coordinated with cell- and molecular biology research.

