Changing Paradigms in Plant Breeding

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The origin of plant breeding traces back to the dawn of agriculture and the domestication of plants, when nomadic man first became a settler. This started in those regions of the world where plants suitable for domestication, such as large grain grasses, grew in the wild. We envisage that the early farmers collected seeds and vegetative reproductive organs (tubers and bulbs) and kept part of them for planting the next season's crop. During this process, they selected naturally occurring variants that were higher yielding and better adapted for cultivation. Selected variants would, for instance, retain their seeds after ripening, have larger and healthier seeds, and carry less thorns or prickles, resulting in what we now sometimes refer to as the "domestication syndrome" of cultivated plants. Uncontrolled hybridization in farmers' fields, as well as with wild relatives and/or progenitors occurring in the natural habitat, most likely increased the variation in the germplasm available to early farmers. Over many centuries, the conscious or unconscious selecting by farmers, along with selective pressures imposed by the temporal and spatial heterogeneity of the growing conditions, resulted in land races, genetically heterogeneous populations that are locally adapted to the conditions imposed by man and the physical environment. The process of crop improvement by farmers' selection, however, was a very slow process compared with science-based professional plant breeding.

The first gradual change toward plant breeding as a specialized profession occurred when private agriculturalists began to deliberately select cultivars and started selling seeds of improved quality. In practice, this form of plant breeding implied the selection of superior variants among existing variation (usually through mass selection and occasionally through line or family selection) and increasing the uniformity of the crop. Until the beginning of the 20th century, controlled hybridization to create novel variation available for selection was rarely involved.

The rediscovery of Mendel's work at the turn of the century provided a solid scientific basis for plant breeding. The awareness of the particulate nature of hereditary "factors" and the possibility to create novel combinations of traits by making crosses contributed enormously to the professional "seedmanship" of the 20th century.

Concepts such as resistance breeding, the introgression of specific traits (often disease resistances) from less-related and often difficult-to-cross species, mutation breeding, hybrid and synthetic varieties, the use of male sterility, and incompatibility to enable the efficient production of such hybrid varieties all emerged before 1975. These developments were largely occurring in the field of plant breeding and plant genetics itself with the help of plant pathologists. Plant physiologists and plant biochemists, with a few exceptions, contributed little to plant breeding practice until 1975.

DEVELOPMENTS IN PLANT BREEDING AFTER 1975

Plant Cell and Tissue Culture

A number of developments in basic plant science started to affect plant breeding from the early 1970s. The first was the further development of plant cell and tissue culture. The use of embryo rescue techniques to achieve hybridization with less-related species started earlier. The introduction of the nematode resistance gene *Mi* from wild tomato (*Lycopersicon peruvianum*) into the cultivated tomato (*Lycopersicon esculentum*) on the basis of an interspecific hybrid obtained by embryo rescue (7) is a very successful illustration of the usefulness of this technology. Improvement of cell culture techniques and the possibility to regenerate plants from a single cell gave the promise of efficient selection at the cell and tissue culture level, especially for traits such as stress tolerance. These expectations have not been met because of several reasons, one of them being that many selected variants turned out not to be of a genetic nature and also because cells in culture do not always behave the same as plants in the field. After the initial surprise that not all plants regenerated from a specific genotype by tissue culture were identical, plant breeders saw this phenomenon as another potential source of useful genetic variation. Larkin and Scowcroft (3)

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coined the term "somaclonal variation" for this, which was almost immediately generally accepted. However, because stable mutations occurring in tissue culture are mostly negative, as are mutations induced by classical mutagens such as irradiation and chemicals, this technique did not provide the extra source of novel and unique genetic variation that some had expected.

Protoplast fusion is another cell and tissue culture technique of which the first papers describing successful experiments were published in the 1970s. The tomato–potato hybrid reported by Melchers et al. (4) was one of the first examples that suggested that this technique could expand the germplasm pools available for breeders. However, it appeared that this technique was of limited value because of problems with somatic incongruity, which did not allow generating hybrids that were sufficiently fertile for further breeding when the parents are too distantly related. An intrinsic novelty of somatic hybridization is the possibility to create novel combinations of organelles such as chloroplasts and mitochondria, which allowed the successful introduction of the mitochondrial-encoded male sterility from *Raphanus sativus* into *Brassica napus* without the unwanted *R. sativus* chloroplasts (5). An important application of tissue culture is the development of haploid induction procedures, either by anther culture or by chromosome elimination. The latter technique also requires tissue culture technology as well as the in vitro maintenance and rapid propagation of breeding material.

The Cloning of Useful Genes and Transgenic Plants

Until recently only those genes available within the germplasm of the crop plant and some related species were available for breeding. However, when transformation procedures were developed that allowed the introduction of DNA into an organism, almost any gene became available. Developments in molecular biology allowed the cloning of specific genes to be used for transformation as well as the control of their expression. The use of *Agrobacterium tumefaciens* as a versatile vector for transformation was an important breakthrough, even more so in the 1990s when it was convincingly shown that it also could be used on cereals, which as the seed legumes had been among the most recalcitrant to transformation. Other technological breakthroughs were the use of cell- and plant-selectable markers and the development of novel transformation techniques (1). The latter techniques include the use of biolistics and simpler techniques for *A. tumefaciens*-mediated transformation such as explant transformation and, for Arabidopsis, the extremely efficient and simple floral dip or vacuum infiltration procedure. Useful genes for plant breeding are already abundant and could be used to solve previously impossible or very difficult-to-solve problems. These include resistance to insects using *Bacillus thuringiensis* genes, resistance to viruses using coat protein immunization, gene-silencing strategies, and an increase in general resistance by the introduction of constitutively systemic acquired resistance. In addition to improving the tolerance and/or resistance to biotic and abiotic stress, genetic modification offers many possibilities for modifying the development of plants and their chemical composition. Examples of developmental changes include the engineering of male sterility, the modification of fruit ripening, and alterations in flowering behavior and plant architecture. The chemical compositions of fruits and seeds can even be modified so that they can produce non-plant compounds such as antibodies and biodegradable plastics (6). The possibility to obtain rice varieties with a high level of vitamin A and a better iron uptake, which could alleviate the nutritional problems of many people, is very appealing (11). Among the novel traits introduced with this technology was herbicide resistance, which raised an emotional aversion against transgenic plants among consumers because they did not want (more) herbicides to be used. Although it is obvious that this technology has tremendous possibilities for plant breeding and human well being, it appears that this is the first time that the introduction of a novel biological technique became the subject of such public scrutiny. It is remarkable that similar concerns about transgenics are virtually absent in the area of medical applications.

Marker-Assisted Breeding

In addition to genetic modification, molecular biology has provided another tool for plant breeders: DNA markers. This tool seems to have provoked fewer disputes among the public, probably because it affects the intrinsic properties of crops less directly. The selection of superior genotypes is often hampered by the significant influence that environmental factors have on the expression of a trait and the variability of these environmental factors. This is especially true for traits related to crop yield. In addition to their sensitivity to environment and the phenomenon of genotype-by-environment interaction, (i.e. the differential reaction of genotypes to environmental changes), such traits are often controlled by a large number of genes. These factors make it difficult to analyze their genetic basis and, therefore, complicate breeding. The efficiency of selection for such traits can be improved when one can monitor the genotype directly. This can be done when one knows either the genes responsible for the traits or genes that are closely linked to them. Many molecular techniques are now available for monitoring such genes. The application of these require: (a) the presence of polymorphisms at the DNA level that

can be analyzed easily and cost effectively (often by using PCR), and (b) knowledge about the genetic location of molecular markers in relation to the traits of interest. The assessment of the approximate map position of the genes responsible for the observed quantitative genetic variation (called quantitative trait loci [QTL]), can be done by scanning the markers on an ordered linkage map for association with trait values in a segregating mapping population. This detailed knowledge of the genetics of complex traits can then be used to select indirectly for the desired characteristics on the basis of markers only.

QTL mapping not only enables localization of polygenes on a linkage map, it also allows the estimation of the effects of individual QTL as well as their joint effects (epistasis). In a number of studies, this approach has revealed the environment dependence of QTL effects, thus helping to elucidate the phenomenon of genotype-by-environment interaction. QTL analysis thus has generated detailed knowledge on the genetics of complex traits and at the same time provided plant breeders with a useful tool for indirect selection. The study of QTL at the molecular level will become increasingly feasible as shown by the recent cloning of a fruit-size-determining gene (Fig. 1) in tomato (2).

Another extremely useful application of marker technology is marker-assisted introgression. The introgression of single genes from exotic germplasm into breeding material by repeated backcrossing is greatly facilitated by the use of markers because the donor and the recipient genome fractions can be monitored in the successive generations of backcrossing. The number of generations required to recover an improved near-isogenic introgression line can thus be reduced by 50%, in comparison with the classical procedure. This "marker-guided introgression" is now being routinely applied in the breeding programs of several crops.

It is not only monogenic traits that are amenable to "guided introgression." By combining the QTL approach with backcrossing, useful genes that control quantitative traits have been identified in the germplasm of plants not adapted to agriculture and have successfully been transferred to advanced breeding lines. The identification of alleles that increase fruit size in the small-fruited wild tomato (*Lycopersicon pimpinellifolium*) is an appealing example of this (9). Stuber et al. (8) similarly were able to create enhanced inbred lines of maize (*Zea mays*) using obsolete inbreds as donors, resulting in F_1 hybrids that significantly outyielded the original hybrid. Tanksley and McCouch (10) have expressed the opinion that "unlocking the genetic potential from the wild" using this approach will be a major tool for future crop improvement. Molecular analysis has revealed that the germplasm of wild relatives, either still available in the wild or conserved in gene banks, is vastly broader than the narrow gene pools of cultivated plant species.

THE FUTURE

The developments described above imply that in plant breeding the paradigm has changed from selection of phenotypes toward selection of genes, either directly or indirectly. Plant breeders try to optimize the use of the genetic variation in nature by bringing together in one genotype alleles that maximize yield, resistance to stress, etc. However, because genes do not function as single entities it is necessary to know how numerous genes function together. This, in turn, requires knowledge of the potential and constraints of biological functions of plants. The understanding of the interaction between genes, organs, and environmental factors, which include other organisms, is a major challenge for plant biologists. To obtain this information, it is important to exploit the

Figure 1. Genetic variation among cultivars and related species of tomato for fruit characteristics which includes variation for size, shape, and color. Variation is shown both for immature fruit color ranging from pale to dark green and for mature fruit color ranging from yellow-green in small-fruited species such as wild tomato (*L. peruvianum*) to red and yellow. Domestication of tomato was accompanied by a dramatic increase in fruit size (compare the small fruits of the wild species and the large fruits of some of the cultivars). Frary et al. (2) demonstrated that one of the genes that controls this quantitative trait could be cloned.

tools of classical and molecular genetics. To these disciplines a set of technologies summarized as "genomics" has recently been added.

Knowledge of the factors that limit the functioning of plants is essential and may be used to design the ideal plant type. When the factors that are limiting the optimal functioning of plants are known, relevant genes can be identified to enable repair of the "defect." Thereafter, such genes could be searched for in the available germplasm. To this end, it is important to have access to all existing genetic variation both within and outside the species. The possibility to transfer genes across almost all taxonomic borders by molecular techniques has expanded the potential resources available to plant breeders enormously. It is becoming generally accepted that a multidisciplinary approach to plant biology will lead to the disappearance of borders between disciplines and the irrelevant difference between classical and modern (molecular) plant breeding. In the same vein, the differences between transgenic and non-transgenic crops should become irrelevant when the focus of plant breeding is on achieving maximal production in a sustainable way to feed the growing human population.

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