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Future of plant biotechnology in crop improvement

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Cultivation of crops having multiple durable resistance to diseases and pests will be made easier by plant biotechnology. Transgenes and marker-assisted selection will aid in the development of high yielding crops, which will be needed to feed the world and save land for the conservation of plant biodiversity in natural habitats. The genetic base of crop production will be conserved and further widened by the integration of biotechnology tools in conventional breeding. Utilization of specific genotypes to particular cropping systems will be facilitated. Value-added high quality crops will be obtained through multidisciplinary collaboration among plant breeders, biotechnologists, natural product chemists and other plant scientists.

Background information

Rapid advances in plant biotechnology make it rather difficult to analyze its future in crop improvement. Within the last 100 years the mankind has witnessed the rise of genetics as a scientific discipline (1900s), the discovery of DNA as the hereditary material (1944), the elucidation of the double helix structure of the DNA molecule (1953), the deciphering of the genetic code (1966), the ability to isolate genes (1973), and the application of DNA recombinant techniques (from 1980 onwards).

Methods of crop improvement evolved dramatically throughout the 20th century. Mass and pure line selection in landraces, consisting of genotype mixtures, were the popular breeding techniques until the 1930s for most crops. In the 1930s maize breeders started the commercial development of double cross hybrids that was followed by the extensive utilization of single crop hybrids. Pedigree-, bulk-, backcross- and other selection methods were also developed especially for self-pollinating crop species (Troyer 1996). Such scientific advances in plant breeding led to the so-called 'green revolution', owing to which cereal production accounting for more than 50% of the total energy intake of the world's poor, kept in pace with the high average population growth rate of 1.8% since 1950 (Daily *et al* 1998). Today, 370 kg of cereals per person are harvested as compared to only 275 kg in the 1950s; i.e., in excess of 33% per capita gain. Similar progress in other food crops resulted in 20% per capita gains since the early 1960s (FAO 1995). There are 150 million fewer hungry people in the world today than 40 years ago, though there are twice as many human beings. Despite such an impressive progress in crop productivity, even greater progress must be made in order to feed an additional two billion people by the early part of the 21st century (Anderson 1996). Around 800 million people are hungry today and another 185 million pre-school children are still malnourished owing to lack of food and water, or disease. Hence as suggested by the Nobel Peace Laureate, Norman Bourlag (1997) new biotechniques, in addition to conventional plant breeding, are needed to boost yields of the crops that feed the world. Careful choice of such biotechniques as well as a realistic assessment of their potential in crop improvement are needed to avoid not only the criticism of the anti-biotechnology lobbyists but also the permanent distrust of pragmatic traditional breeders.

Modern biotechnology

Tissue culture, developed in the 1950s, became popular in the 1960s. Today, micropropagation and *in vitro* conservation are standard techniques in most important crops. At the beginning of the 1980s genetic engineering of plants remained a promise of the future, although gene transfer had already been achieved earlier in a bacterium. The first transgenic plant, a tobacco cultivar resistant to an antibiotic, was reported in 1983. Transgenic crops with herbicide, virus or insect resistance, delayed fruit ripening, male sterility, and new chemical composition have been released to the market in past decade (NCGR 1998). In 1996, there were about 3 million ha of transgenic crops grown in the world (mainly in North America) whereas an excess of 34 million ha (a 12-fold addition) of transgenic crops were supposedly harvested this year in North America, Argentina, China, and South Africa among other countries. Argentina is the leading developing country with an excess of 4 million ha of transgenic herbicide-resistant soybean. There are more than 4.4 million ha of transgenic corn (14% of total acreage), 5 million ha of transgenic soybean (20%), and 1.6 million ha of transgenic canola (42%) grown only in North America. It has been calculated that in 1998 US farmers grew over 50% of their cotton fields with transgenic seeds, the largest percentage for any crop ever. Trees are the next target in the agenda of genetic engineering.

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Genetic markers

In 1950s allozymes were employed as the first biochemical genetic markers. Population geneticists took advantage of such marker system for their early research. In the 1970s, restriction fragment length polymorphisms (RFLP) and Southern blotting were added to the toolbox of the geneticists. Taq polymerase was found in the 1980s, and the polymerase chain reaction (PCR) developed shortly afterwards. Since then, marker-aided analysis based on PCR has become routine in plant genetic research and marker systems have shown their potential in plant breeding (Paterson 1996). Furthermore, new single nucleotide polymorphic markers based on high-density DNA arrays (Chee *et al.* 1996), a technique known as 'gene chips', have recently been developed (Lemieux *et al.* 1998, Marshall and Hodgson 1998, Ramsay 1998). With 'gene chips', DNA belonging to thousand of genes can be arranged in small matrices (or chips) and probed with labeled cDNA from a tissue of choice. DNA chip technology uses microscopic arrays (or micro-arrays) of molecules immobilized on solid surfaces for biochemical analysis. An electronic device connected to a computer may read this information, which will facilitate marker-assisted selection in crop breeding. In summary, since Mendel's work on peas, there have been five eras in genetic marker evolution: morphology and cytology in early genetics (until late 1950s), protein and allozyme electrophoresis in the pre-recombinant DNA time (1960 - mid 1970s), RFLP and minisatellites in the pre-PCR age (mid 1970s - 1985), random amplified polymorphic DNA, microsatellites, expressed sequence tags, sequence tagged sites, and amplified fragment length polymorphism in the oligoscene period (1986 - 1995), and complete DNA sequences with known or unknown function as well as complete protein catalogs in the current computer robotic cyber genetics generation (1996 onwards).

Bioinformatics

Development of fast and more dependable computers has allowed easier management and analysis of data as well as publication of scientific reports. More publications and easy means for retrieval of this information have brought about phenomenal growth of knowledge dissemination in plant genetics and breeding. Rapid information exchange has been further facilitated by electronic mail and access to the internet. Information technology and biotechnology are beginning to merge into the new field of 'bioinformatics'. Scientists working in this field are developing biological data banks, which can be downloaded through internet by other scientists.

Crop genomics

This new term refers to the investigations of whole genomes by integrating genetics with informatics and automated systems (Briggs 1998). Genomic research aims to elucidate the structure, function and evolution of past and present genomes. Some of the most dynamic fields concerning agriculture are the sequencing of plant genomes, comparative mapping across species with genetic markers, and objective assisted breeding after identifying candidate genes or chromosome regions for further manipulations. As a result of genomics, the concept of gene pools has been enlarged to include transgenes and native exotic gene pools that are becoming available through comparative analysis of plant biological repertoires (Lee 1998). Understanding the biological traits of one species may enhance the ability to achieve high productivity or better product quality in another organism.

DNA markers and gene sequencing provides quantitative means to determine the extent of genetic diversity and to establish objective phylogenetic relationships among organisms. 'Gene chips' and transposon tagging will provide new dimensions for investigating gene expression. Molecular biologists will study not only individual genes but how circuits of interacting genes in different pathways control the spectrum of genetic diversity in any crop species. For example, more information will be available on why plant resistance genes are clustered together, or what candidate genes should be considered when manipulating quantitative trait loci (QTL) for crop improvement (Paterson 1997, Liu 1997).

Functional genomics

Genomics may provide a means for the elucidation of important functions that are essential for crop adaptedness. Regions of the world should be mapped by combining data of geographical information systems, crop performance, and genome characterization in each environment. In this way, plant breeders can develop new cultivars with the appropriate genes that improve fitness of the promising selections. Fine-tuning plant responses to distinct environments may enhance crop productivity. Development of cultivars with a wide range of adaptation will allow farming in marginal lands. Likewise, research advances in gene regulation, especially those processes concerning plant development patterns, will help breeders to fit genotypes in specific environments. Photoperiod insensitivity, flowering initiation, vernalization, cold acclimation, heat tolerance, host response to parasites and predators, are some of the characteristics in which advanced knowledge may be acquired by combining molecular biology, plant physiology and anatomy, crop protection, and genomics. Multidisciplinary co-operation among researchers will provide the required holistic approach to facilitate research progress in these subjects.

Gene banks and plant breeding

The sequencing of crop genomes opened new frontiers in conservation of plant biodiversity and its genetic enhancement. The advances in gene isolation and sequencing in many plant species allow to envisage that within a few years, gene-bank curators may replace their large cold stores of seeds with crop DNA sequences that will be electronically stored. The characterization of plant genomes will ultimately create a true gene bank, which should possess a large and accessible gene inventory of today's non-characterized crop gene pools. Of course, seed banks of comprehensively investigated stocks should remain because geneticists and plant breeders, the main users of gene banks, will need this germplasm for their work. Genomics may accelerate the utilization of candidate genes available at these gene banks through transformation without barriers across plant species or other living kingdoms. Nonetheless, genetic engineering should be seen as one of the methods of plant breeding that permits the direct alteration and re-building of a crop population. "Shutting-off" genes coding for undesired characteristics may be another application of transgenics in crop improvement.

Plant breeders will change their *modus operandi* with the development of objective marker-assisted introgression and selection methods. Backcross breeding will be shortened by eliminating undesired chromosome segments (also known as linkage drags) of the donor parent or selecting for more chromosome regions of the recurrent parent. Parents of elite crosses may be chosen based on a combination of DNA markers and phenotypic assessment in a selection index, such as best linear unbiased predictors. To achieve success in these endeavors, cheap, easy, decentralized, and rapid diagnostic marker procedures are required.

Bioresource technology

Nowadays, the finding of new genes that add value to agricultural products seems to be very important in the private sector. Unique gene databases are being assembled by the industry with the massive amount of data generated by genomics research. A new term 'biosource' has been coined recently to refer to a fast and effective technology of pinpointing genes. With this method, a 'benign' virus infects a plant with a specific gene that allows researchers to observe directly its phenotype. Biosource replaces the standard time-consuming approach of first mapping a gene to subsequently determine its exact function. Gene identification in DNA libraries coupled with biosource technology and an enhanced ability to put genes into plants will be routine for improving crops in the next decade.

Farming and pharming

The aims of applied plant science research for agriculture are to enhance crop yields, improve food quality, and preserve the environment where human beings and other organisms live. The best way for conservation of plant biodiversity and its environment, would be to achieve high crop productivity per unit area. In this regard, it has been reported that as yields treble, soil erosion per ton of food decreases by two-thirds. There has been a significant yield improvement owing to enhanced crop husbandry, but in the next years progress will be achieved by changing plants that could be more suitable to sustainable and environmentally friendly farming systems.

In the next decades meiotic-based breeding will still generate cultivars for farmers. Genetic improvement through biotechnology needs conventional breeding because (1) the elite cultivars will be the parents of the next generation of improved genotypes, (2) field testing across locations or cropping systems and over years will be needed to determine the best selections due to the genotype-by-environment interaction. Transgenes must be viewed as improvements rather than replacements for elite germplasm. Indeed, genetic engineering may provide a means to add value by introducing synthetic or natural genes that enhance crop quality and yield, as well as protect the plant against pest and diseases. Farmers will pay more for transgenic crop propagules if they obtain extra-income after adopting biotech-derived products. For example, seeds of insect resistant transgenic crops will be more expensive than those of available cultivars but the farmer will not need to apply pesticides in their transgenic fields. Of course, patents make transgenic seeds more expensive but also farmer's benefits may be higher.

Growth of cities is rapidly replacing farmland with shopping complexes, parking lots, and housing developments. Peri-urban agriculture and home gardening are also becoming very important for national food security in the developing world as a result of rapid urban expansion. Hence, new cultivars will be needed to fit into intensive production systems, which may provide the food required to satisfy urban world demands of the next century. Specific plant architecture, tolerance to urban pollution, efficient nutrient uptake, and crop acclimatization to new substrates for growing are, among others, the plant characteristics required for this kind of agriculture. Genes controlling these characteristics may be available in gene banks for further cross breeding, which can be assisted by genomics. Peri-urban and home garden 'farmers' will have to adapt to new demands from emerging urban populations with higher income. These consumers may request a more varied diet. For example, food crops with low fats, and high in specific amino acids may be needed to satisfy people who wish to change their eating habits. If genes controlling these characteristics do not exist in a specific crop pool they may be incorporated into the breeding pool using transgenics.

Often plants provide the raw materials for agro-industry, and not only for food or fibre processing. Active ingredients of plants have been transformed into commercial products such as medicines, solvents, dyes, and non-cooking oils for many years. Hence, it would not be surprising to see, in few years from now, entire farms without food crops but growing transgenic plants to produce new products, e.g. edible plastic from peas or plant oils to manufacture hydraulic fluids and nylon. This new rural activity may result in important changes in the national economic sector.

'Pharming' indicates a new kind of system to obtain medicines (Anderson 1996). For example, oral vaccines appear to be a convenient delivery system for vaccination throughout the world. Biotechnology has been used to engineer plants that contain a gene derived from a human pathogen (Tacker *et al.*

1998). An antigenic protein encoded by this foreign DNA can accumulate in the resultant plant tissues. Results from preclinical trials showed that antigenic proteins harvested from transgenic plants were able to keep the immunogenic properties if purified. These antigenic proteins caused the production of specific antibodies in injected mice. Mice, which ate these transgenic plant tissues, also showed a mucosal immune response. The ability of transgenic food crops to induce protective immunity in mice against cholera toxin has been recently demonstrated (Arakawa *et al.* 1998). Potato tubers have been used successfully as a biofactory for production of recombinant single chain antibody (Artsaenko *et al.* 1998).

Epilogue

Banning transgenic crops in the farming system will be foolish because the potential benefits are so great. Whatever scientists do to develop crops that eliminate or reduce the utilization of polluting agro-chemicals in the farming systems must be welcome by farmers and consumers. The general public should not fear biotechnology, rather consider it as a safe tool for scientific crop improvement, because it helps in the fight against hunger and poverty. Therefore, research funding should be allocated accordingly to long-term plant breeding programs, which include biotechnology as one of its tools. In this way, we may effectively face the serious challenge of feeding the rapidly growing world population in this millennium.

Within the next 10 or 20 years, five research areas may become very important for crop improvement: (i) apomixis to fix hybrid vigour, (ii) male sterility systems with transgenics for hybrid seed in self-pollinating crops, (iii) parthenocarpy for seedless vegetables and fruit trees, (iv) short-cycling for rapid improvement of forest and fruit trees, and (v) converting annual into perennial crops for sustainable agricultural systems. The development of perennial crops will be especially important to protect the soil from erosion. Plant biotechnology will play, of course, an important role in achieving research and development success in these areas. ■

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