BIOTECHNOLOGY FOR FOOD: KEY TOPICS IN PLANT BIOTECHNOLOGY

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Abstract

Agricultural production is becoming increasingly knowledge-based and science-intensive. Rapidly expanding research in biological control, management of genetic resources, natural fertilization and agro-ecology profoundly affect the capacity to produce food. Today, biotechnology is being used as a tool to give plants new traits that benefit agricultural production, the environment, and human nutrition and health. This review focuses on key topics in agricultural biotechnology with an emphasis on Genetics and breeding, Inputs, outputs and value added traits , Products and applications, Plant development, and Environment
Keywords: *biological control, biotechnology, food*

Introduction

Plants and agriculture have played an important role in the development and advancement of civilization. Plants provide sustainable supplies of food for humans and animals, fiber for construction and clothing, medicines and drugs, perfumes, chemicals for industrial processes, energy and, most recently, biomass to meet the increasing demand for fuels. Plants also play a major environmental role by preventing soil erosion, increasing levels of oxygen in the atmosphere, reducing carbon dioxide emissions, and enriching soils with nitrogen.

In 2007, the world population had reached 6.6 billion (UNFPA, 2007), and the latest revised forecast for world population estimates for the year 2050, 9.4 billion people (Census, 2007), while agricultural production is growing at the slower rate of about 1.8 % annually. All human beings depend on agriculture that needs to produces food of the appropriate quality and at the required quantities. The 21st century technological revolution created by genomics, generate biotechnologies which provides a unique opportunity to achieve this goal (Meiri, 1998). The United Nations Convention on Biological Diversity defined biotechnology as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use"(CBD, 1992). Genetically engineered crops are delivering benefits through more affordable food, feed, and fibber that require fewer fertilizer and pesticide applications, produce vaccines to prevent major communicable diseases, have improved tolerance to drought, heat, and cold, conserve more soil, and provide for a more sustainable environment.

During the last two decades, new biotechnologies have been adapted to agricultural practices and have opened new vistas for plant utilization. Plant biotechnology (especially *in vitro* regeneration and cell biology, DNA manipulation and genetic modification of biochemical pathways) is engineering the plant by controlling growth and development, by increasing resistance to threats of abiotic and biotic stress and by expanding the horizons by producing specialty foods, biochemicals and pharmaceuticals (Altman, 1999). From a more synthetic perspective, the key topics in Plant biotechnology are: Genetics and breeding, Inputs, outputs and value added traits, Products and applications, Plant development and Environment (EUFIC).

Experimental

This key topic comprises two main interrelated area of research: *genome research* and *genetic markers in breeding*.

Genomic research seeks to understand the structure, evolution and function of genes and genomes. Application of genomics to study the crop species offers special opportunities for innovative approaches, for combining information on DNA sequences with their function. The knowledge of plant genomes is required to accelerate the process of plant improvement, greater assurance of food security, extended uses of plant products and to enhance the utility and value of crop plants beyond traditional uses.

In 1990, the Multinational Coordinated *Arabidopsis thaliana* Genome Research Project was launched by an international group of scientists who recognized the need to examine in detail one simple plant with basic features common to all plants. *Arabidopsis thaliana* is a small plant in the mustard family, and has the smallest genome and the highest gene density so far identified in a flowering plant. On 14th of December 2000, *Nature* report the first complete genome sequence of a plant as a common effort of ***Arabidopsis* Genome Initiative,** for more comprehensive comparison of conserved processes in all eukaryotes, identifying a wide range of plant-specific gene functions and establishing rapid systematic ways to identify genes for crop improvement (AGI, 2000). This was one of the major achieving in plant genomics. The availability of the complete genome sequence of *Arabidopsis thaliana* provided a "quantum leap" in the information base for the next approach: functional genomics. The term functional genomics can be referred to as the "development and application of global (genome-wide or system-wide) experimental approaches to assess gene function by making use of the information and reagents provided by structural genomics" (Hieter, 1997).

A 10-year functional genomics research program initiated in 2001 represent a long-range plan for a new phase of the *Arabidopsis* Genome Research Program. The goals of this project are to determine the function of every *Arabidopsis* gene and obtain a detailed and comprehensive understanding of the molecular processes underlying the development, metabolism and interaction with the environment of a flowering plant. *Arabidopsis* research has provided the cutting edge in generating resources and analytical tools, providing an example for the investigation of other plant species.

The second major achieving in plant genome research was reported in December 2002 by IGRSP, a consortium of public sequencing teams from around the world, by announcing that rice genome has successfully been sequenced (IRGSP, 2002). The rice genome sequence is fundamental for development new improved types of rice.

It will also expand knowledge about crop yield, disease and pest resistance, hybrid vigour and adaptability to different environmental conditions. Because rice is a model cereal for genome sequencing and basic research, (rice has the smallest genome of the major cereals, which include corn, wheat, rye, barley, oats, millet and sorghum), the completion of its genome is a key to understanding the genomic structure and for the improvement of other grasses.

By date only a few plant species have the entire genome sequenced; a list of the complete sequenced plant genomes can be followed in table 1.

A number of other major crop plants are in process of complete genome sequencing: maize, wheat, barley, tomato, sorghum and alfalfa. Concurrently have been developed extensive databases (PlantGDB, MaizeGDB, NCBI, TAIR, TIGR, SIGnAL, etc.) of EST, cDNA and protein sequences to asses genes structure and function, for the most plant species. EST sequencing and mapping or genomic sequencing of a few strategic crop species may suffice to facilitate the transfer of information from model species to the majority of crop plants, to be used for crop improvement in the future.

Table 1: Complete sequenced plant genomes

Organism	Type	Relevance	Genome size	Number of genes predicted	Organization	Year of com-pletion
Arabidopsis thaliana Ecotype: Columbia	Wild mustard	Model plant	120 Mb	25.498	Arabidopsis Genome Initiative	2000
Cyanidioschyzon merolae Strain:10D	Red alga	Simple eukaryote	16.5 Mb	5.331	University of Tokyo, Rikkyo University, Saitama University and Kumamoto University	2004
Oryza sativa **ssp indica**	Rice	Crop and model organism	420 Mb	32-50,000	Beijing Genomics Institute, Zhejiang University and the Chinese Academy of Sciences	2002
Oryza sativa **ssp japonica**	Rice	Crop and model organism	466 Mb	46,022-55,615	Syngenta and Myriad Genetics	2002
Ostreococcus tauri	Green alga	Simple eukaryote	12.6 Mb		Laboratoire Arago	2006
Populus trichocarpa	Balsam poplar or Black Cottonwood	Carbon sequestration, model tree, commercial use (timber), and comparison to *A. thaliana*	550 Mb	45.555	The International Poplar Genome Consortium	2006

Genetic markers are known DNA sequences that flag the presence of genes that control particular traits and can be identified by a simple assay. Molecular markers are used to identify the presence of a gene directly from a sample of plant without resorting to the more lengthy process of screening for physical and chemical characteristics. The information gained is used for *marker assisted selection* or for *genetic engineering.*

Marker assisted selection (MAS) can be applied to replace evaluation of a trait that is difficult or expensive to evaluate. When a marker is found that co-segregates with a major gene for an important trait, it is easier to screen for the presence of the marker allele linked to the gene, than to evaluate the trait (Francia, 2005). Successful practical application of marker-assisted selection was described in tomato (Breto, 1994), forage grasses (Hayward, 1994) apple (Gianfranceschi, 1996), rice (Huang ,1997), maize (Sari-Gorla, 1997), sunflower (Lawson, 1998), barley (Romagosa, 1999), cassava (Mba, 2001), wheat (Ellis, 2002), rye (Stracke, 2003) cucumber (Fazio, 2003), cotton (Zhang, 2003), soybean (Spencer, 2004), carrot (Yau, 2005), potato (Gebhardt, 2006), bean (Namayanja, 2006), oilseed rape (Nath, 2007), diploid alfalfa (Narasimhamoorthy, 2007), and the list could be extended to many other crop species.

Genetic engineering is the process of removing, modifying or adding genes to a strand of DNA with the purpose of producing new substances or improving functions of existing organisms.

The list of plants and plant-derived products made as a result of modern biotechnology is ever increasing (table 2). Genetically modified (GM) crops have spread faster in the past decade than any agricultural technology. For example, in US from the nearly 250 million acres of GM crops planted in 2006, about 173 million acres of maize, cotton and soybeans, among others, have been genetically modified to resist the herbicide glyphosate (Vines, 2001). A "News Focus" story in the May 25 issue of the journal *Science* reported a similar situation for Europe: "... genetically modified crops are flourishing worldwide, including in six European Union countries. Last year (2006), 10 million farmers in 22 countries planted more than 100 million hectares with GM crops" (Service, 2007).

Some of the potential **benefits** from using transgenic plants include:
Reduced crop production costs and increased yields:

- Healthier, more nutritious foods
- Reduced environmental impact from farming and industry
- Increased food availability for underdeveloped countries

Potential **risks** associated with transgenic plants include:

- Introduction of allergenic or otherwise harmful proteins into foods
- Transfer of transgenic properties to viruses, bacteria, or other plants
- Detrimental effects on non-target species and the environment

Table 2: Timeline of Plant Biotechnology

Year	**Event**
1700s	Naturalists identify hybrid plants
1860s	Austrian botanist Gregor Mendel studies pea plants and recognizes that specific traits are passed from parents to offspring – these traits are eventually discovered to be genes
1900	European botanists begin to improve plant productivity using genetic theories based on Mendel's work
1922	Farmers purchase hybrid seed corn created by crossbreeding two corn varieties
1953	Structure of DNA is discovered – marking the beginning of modern genetic research
1970s	Hybrid seeds are introduced to developing countries to increase food supplies
1973	Genetic engineering is used to precisely manipulate bacterial DNA
1983	First GM plant is created; a tobacco plant resistant to an antibiotic
1985	GM plants resistant to viruses, bacteria, and insects are field tested
1986	EPA approves the release of the first GM crop (herbicide resistant tobacco)
1990	First successful field trial of GM cotton (herbicide resistant)
1992	FDA decides GM foods will be regulated as conventional foods
1994	FlavrSavr Tomato becomes the first GM food to be approved for sale
	Transgenic Pea Seeds expressing resistance to Bruchid Beetles
1995	Herbicide resistant canola and corn
2000	Herbicide resistant cotton, soybeans, sugar beet as well as insect or virus resistant corn, cotton, papaya, potato, squash, tomato approved in the U.S.
2001	"Golden rice" which may help prevent cases of blindness and death caused by Vitamin A and iron deficiencies
2002	Transgenic wheat expressing resistance to mosaic virus
	Transgenic sugarcane expressing resistance to sugarcane mosaic virus, strain E
	Transgenic *Prunus dulcis* expressing resistance to prune dwarf virus
	Fungal resistant sunflower
2003	Transgenic cotton expressing enhanced fungal resistance
	Transgenic tobacco that express enhanced photosynthesis and growth
	Transgenic cucumber fruits that produce elevated level of an anti-aging superoxide dismutase
2004	Transgenic melon salt tolerant
2005	Transgenic eggplant resistant to fruit and shoot borer
2006	Super-sized cassava plants
	Herbicide resistant oilseed rape
2007	Transgenic alfalfa, which accumulates resveratrol
Jan	Release of dicamba resistance technology to soybean and other broadleaf plant species (Behrens, 2007)
May	Release of engineered oilseed rape with increased seed oil content
	Transgenic tomato that express significantly increase size of flower and fruits
	African produced GE maize, resistant to the maize streak virus, is evaluated
July	

➢ *Inputs, outputs and value added traits*

Changes made to plants through the use of biotechnology, can be categorized into the three broad areas of *input, output,* and *value-added traits.*

Input traits. An "input" trait helps producers by lowering the cost of production, improving crop yields, and reducing the level of chemicals required for the control of insects, diseases, and weeds. Input traits that are commercially available or being tested in plants:

- Resistance to destruction by insects
- Tolerance to broad-spectrum herbicides
- Resistance to diseases caused by viruses, bacteria, fungi, and worms
- Protection from environmental stresses such as heat, cold, drought, and high salt concentration

Output traits. An "output" trait helps consumers by enhancing the quality of the food and fiber products they use. Output traits that consumers may one day are able to take advantage of:

Nutritionally enhanced foods that contain more starch or protein, more vitamins, more anti-oxidants (to reduce the risk of certain cancers), and fewer trans-fatty acids (to lower the risk of heart disease)

Foods with improved taste, increased shelf-life, and better ripening characteristics

Trees that make it possible to produce paper with less environmental damage

Nicotine-free tobacco

Ornamental flowers with new colors, fragrances, and increased longevity

"Value-added" traits. Genes are being placed into plants that complete change the way they are used. Plants may be used as "manufacturing facilities" to inexpensively produce large quantities of materials including:

- Therapeutic proteins for disease treatment and vaccination
- Textile fibers
- Biodegradable plastics
- Oils for use in paints, detergents, and lubricants

Plants are being produced with entirely new functions that enable them to do things such as detect and/or dispose of environmental contaminants like mercury, lead, and petroleum products.

Plants with "input traits" that are commercially available include:

- Roundup Ready® soybean, canola, and corn: resistant to treatment with Roundup herbicide that may result in more effective weed control with less tillage, and/or decreased use of other, more harmful herbicides
- YieldGard® corn and Bollgard® cotton: express an insecticidal protein that is not toxic to animals or humans which protects the plant from damage caused by the European corn borer, tobacco budworm, and bollworm
- Destiny III® and Liberator III® squash: resistant to some viruses that destroy squash.

Results and Discussions

The main plants products that biotechnology is focused on are: glucids (glucose, fructose, sucrose, starch, etc.), oils (essential and fixed oils), storage proteins (albumins, globulins, prolamins, and glutelins) and secondary metabolites (alkaloids, terpenoids, polyketides, glicozides, phenanzines, phenols, etc.). The engineering strategy is to enhance the quantity or the quality of these output traits.

Transgenic plants with engineered output traits, commercially available include:

- High laurate canola and high oleic soybean having altered oil content to be used primarily in industrial oils and fluids rather than food
- High-starch potatoes that take up less oil when frying
- Longer shelf-life bananas, peppers, pineapples, strawberries, and tomatoes
- Vistive™, Low-Linolenic, Soybeans, feeding consumer demand for heart-healthy diets
- Soybeans with higher levels of isoflavones; compounds beneficial in reducing some cancers and heart disease
- Plants that produce vaccines and pharmaceuticals for treatment of human diseases
- Processor Preferred corn hybrids yield more of high extractable starch and include high fermentable corn
- Transgenic maize with increased levels of the amino acid lysine and protein for animal feeds

Applications are wide ranged and include: meal, human health (pharmaceutics, antiseptics), perfumes, cosmetics, flavors, paint industry, leather and textile industry, etc.

➢ *Plant development*

The better insight into the control of plant development (regeneration, morphogenesis, cell division, etc.) achieved during the last two decade, is due to three major findings: the totipotency and regeneration ability of plant cells and tissues, (as revealed by cell culture and micropropagation), the isolation and characterization of genes responsible for hormone production and activation in plants, and the elucidation of pathways and molecular control of the cell cycle and cell signaling (Leyser, 2003). These have enabled both the control and biotechnological manipulation of vegetative growth, generative patterns and of micropropagation. Molecular hormone and cell-cycle research will shed light to a better understanding of vegetative growth patterns.

Thus, the possibility of biotechnologically manipulating plant growth rate and architecture can become a reality. For example, potential consequences of controlled auxin overproduction and availability include: adventitious root formation of importance to propagation, cell and organ elongation for biomass production, increased apical dominance of importance to timber production, etc., (Teale, 2006)

Controlled cytokinin overproduction and availability can result, in enhanced bud break, which is of great importance to plant architecture, branching and compactness, a desired characteristic for some ornamentals, and delayed leaf and plant senescence. No less important, is the potential of affecting the orientation and rate of cell division, cell elongation and tissue longevity, by interfering with the cytoskeleton and cell cycle, the synthesis of cellulose and other cell components, and programmed cell death, respectively (Carles, 2003). A few of these possibilities have already been realized.

Flowers, fruits and seeds are extremely important for agriculture. Biotechnological research aims to control their development and characteristics, and some of the many related studies have already produced practical applications. The major targets in flower development are color, scent and senescence. Strategies for the molecular breeding of flower color and scent include over- and underexpression of color (anthocyanins and carotenoids) and scent (volatiles) compounds, with respect to their biosynthesis and cellular transport. Important targets for controlling fruit development include growth, ripening and senescence, color, scent and, flavor, and particularly metabolic control of sugar, acid and other flavor components. Of great importance to fruits are biotechnological strategies for the production of seedless fruits via parthenocarpy (overproduction of auxin), pollen destruction (no fertilization), or stopping the embryo development.

The manipulation of seed development using biotechnological strategies is of special importance, since the seed industry (together with vegetative propagation material) constitutes the germplasm of the future for any type of plant production system. Seeds and vegetative propagules are packages of genes that form the basis of all advanced and economically viable agricultural industries. Biotechniques and molecular strategies are now available for the major seedbased operations: hybrid seed production, generation of artificial seeds (coated somatic embryos), and for the establishment of germplasm banks that may solve some of the biodiversity issues.

Micropropagation is used routinely to generate a large number of high-quality clonal agricultural plants, including ornamental and vegetable species, and in some cases also plantation crops, fruits and vegetable species. Micropropagation has significant advantages over traditional clonal propagation techniques. These include the potential of combining rapid large-scale propagation of new genotypes, the use of small amounts of original germplasm (particularly at the early breeding and transformation stage, when only a few plants are available), and the generation of pathogen-free propagules.

Particularly noteworthy are the many recent studies on the molecular of organogenesis and somatic embryogenesis. However, further practical applications of micropropagation, which is also commercially viable, depends on reducing the production costs such that it can compete with seed production or traditional vegetative propagation methods (Altman, 1997).

Techniques that have the potential to further increase the efficiency of micropropagation, but still await further improvements, include: simplified large-scale bioreactors, cheaper automatization facilities, efficient somatic embryogenesis and synthetic seed production, greater utilization of the autotrophic growth potential of cultures, and good repeatability and quality assurance of the micropropagated plants.

➢ *Environment*

While plant biotechnology has been applied successfully to fighting a large number of pests, this is not yet the case for abiotic stress conditions such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress. The environmental stress of salinity and drought reduce growth and agricultural productivity more than any other factors.

Drought and salinization are the most common natural causes of lack of food and famine in arid and semiarid regions, and the most serious environmental threats to agriculture in many parts of the world. Desertification, resulting from overexploitation, is often aggravated by regional climatic changes, and results in increased soil erosion and a decrease in land and agricultural productivity.

Land use changes have intensified the use of natural resources and exacerbated many of the processes of land degradation. By the 2050's, 50% of agricultural lands are very likely to be subjected to desertification and salinization, affecting 50% of agricultural lands (FAO, 2007).

Furthermore, the combined effects of climate change and land-use change on food production and food security are related to a larger degradation of lands and a change on erosion patterns.

Although more difficult to control and engineer than the usually monogenic traits of resistance to biotic pests and herbicides, the genetically complex response to abiotic stress is globally and regionally far more important. Therefore, breeding for plant tolerance to drought and salinity stress should be given a high research priority in all future agbiotech programs.

Strategies for the manipulation of osmotic stress tolerance in plants might include: expression of osmoprotectants and compatible solutes, ion and water transport and channels, expression of water-binding and membrane-associated dehydrins and other proteins, transcription factors and DNA-binding proteins, etc. Also of specific interest are the intervening stages of stress perception, signal transduction (ABA and others), and protein modification.

The discovery of new stress-related genes and the design of stress-specific promoters are equally important.

Promising results in manipulation of stress tolerance were reported for tobacco as model plant (Holmström, 2000), and also for *Brassica napus* (Zhang, 2001), wheat (Abebe, 2003), rice (Ren, 2005), oat (Oraby, 2005) and cotton (Zhang, 2007).

Conclusions

The intensification of agriculture cannot be achieved without supporting advanced research and development in biochemistry, physiology, genomics and biotechnology of agricultural plants. Traditionally, agriculture was targeted to improving the production of plant-derived food, in terms of both quantity and quality. This was also the initial primary target of plant biotechnology. The second phase of plant biotechnology is now gradually being implemented: a shift from the production of low-priced food and bulk commodities to high-priced, specialized plant-derived products.

Plant scientists now have a central role in society for the benefit of agricultural production, environment, and human nutrition and health.

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