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REVIEW ARTICLE

Plant biotechnology in the 21st century: the challenges ahead

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In a world where population growth is outstripping food supply agricultural –and especially plant-biotechnology, needs to be swiftly implemented in all walks of life. Achievements today in plant biotechnology have already surpassed all previous expectations, and the future is even more promising. The full realisation of the agricultural biotechnology revolution depends on both continued successful and innovative research and development activities and on a favourable regulatory climate and public acceptance. Biotechnology should be fully integrated with classical physiology and breeding: (1) as an aid to classical breeding, (2) for generation of engineered organisms, (3) for integration of microorganisms into agricultural production systems. Biotechnology is nowadays changing the agricultural and plant scene in three major areas: (1) growth and development control (vegetative, generative and reproduction/propagation), (2) protecting plants against the ever-increasing threats of abiotic and biotic stress, (3) expanding the horizons by producing specialty foods, biochemicals and pharmaceuticals.

The agricultural scene: Food security and the changing environment

In a world where population growth is outstripping food supply agricultural- and especially plant-biotechnology, needs to be swiftly implemented in all walks of life. The world population is expected to reach 7 billion within 25 years, over 10 billion in the year 2050, while agricultural production is growing at the slower rate of about 1.8 % annually. All human beings depend on agriculture that produces food of the appropriate quality at the required quantities.

Since this can no longer be achieved by traditional methods alone, *breeding through plant biotechnology is a necessity*. In the long run the massive and immediate implementation of plant and agricultural biotechnology is more highly crucial than that of medical biotechnology, since more people worldwide die from famine and diseases related to malnutrition than from "modern", western diseases. With "Disaster Management" becoming a central issue in modern sociology research and curricula, *food and food product shortages are the ultimate disaster*. However, unlike most natural disasters, this is one that we can prepare for and even prevent.

Domestication of plants and animals found in the wild, combined with gradual long-term changes in their qualitative and quantitative traits, were the first attributes of agriculture. Domestication, followed by food storage, coincided with the growth of microorganisms. Thus was born classical food fermentation, the earliest known application of biotechnology for the generation of food products. This traditional agriculture now faces several serious limitations:

1. Market limitations: The world is becoming a global village whose free-market rules negate the effectiveness of local pricing policies, and where a dictate of international trade and policies exists. This has greatly affected future developments in agriculture, still the world's largest business.

2. Limitations of natural resources: Global climatic changes (resulting mainly in desertification and salinization), industrialisation and urbanisation, have reduced land and water availability and caused alarming deterioration of soil, water and air quality.

3. Inherent biological genetic limitations: Although previously highly efficient, the release of new improved genotypes by classical breeding is now too slow to cope with the demands, and is considerably limited by the lack of appropriate "natural" genes that can be introgressed by traditional genetic crosses.

Only two major potential solutions seems to exist for increasing food supply and agricultural commodities, in addition to continuously improving agricultural practices, despite the aforementioned limitations: (1) a search for alternative food sources (e.g., marine or extraterrestrial products), (2) enhanced efficient plant breeding.

The need for integration: Combining biotechnology with classical physiology and breeding

Food production, for both quantity and quality, as well as for new plant commodities and products, in developed and developing countries around the globe, cannot rely solely on classical agriculture. Human survival, vis-à-vis a continuous increase in agricultural productivity, depends on the effective *merging of classical breeding with modern plant biotechnology* and the novel tools it provides.

The "green revolution", for example, increased wheat production 10-fold in India and several other countries in South East Asia, thereby feeding three times as many people. However, this revolution has already been exploited to its limits, and alternative solutions are required to breed improved crops. Now biotechnology, integrated with classical breeding, is on the verge of creating the "evergreen revolution".

The potential to improve plant and animal productivity and their proper use in agriculture relies largely on newly developed *DNA biotechnology and molecular markers*. These techniques enable the selection of successful genotypes, better isolation and cloning of favourable traits, and the creation of transgenic organisms of importance to agriculture. Together, these generic techniques are both an extension and an integral part of classical breeding, contributing successfully to shortening breeding and selection cycles.

The new plant biotechnology implies the use of recombinant DNA techniques and in vitro cell biology in three major areas:

1. As an aid to classical breeding: This includes the ongoing genome mapping projects, e.g., in Arabidopsis, rice, maize and tomato, combined with the recent activities in functional genomics, proteomics and bioinformatics, and DNA marker-assisted selection. The combined use of these techniques will soon shorten the time required for "classical" breeding and selection cycles.

2. Generation of engineered (transgenic) organisms: In view of the inherent limitations of introgressing new genes by traditional genetic crosses (i.e., lack of appropriate desired genes and crossing barriers), the efficient engineering of plants has already resulted in improved field-grown transgenic plants in several important crops. The result of this impressive development, which began only 18 years ago, have made possible the direct insertion and integration of genes isolated from several organisms, and the creation of novel, and otherwise impossible genetic recombinations.

3. Integration of microorganisms into plant production systems: The biotechnological development of new symbiotic, antibiotic, and antagonistic relationships between plants and microorganisms (fungi, bacteria and insects) using, among other techniques, engineered plants and microorganisms, creates new possibilities. Some of these include biological control of pests, biofertilization and plant growth stimulation, and bio- and phytoremediation.

During the last two decades, these new biotechnologies have been adapted to agricultural practices and have opened *new vistas for plant utilisation*. This will continue and intensify in the next decade. Plant biotechnology -- especially in vitro regeneration and cell biology, DNA manipulation and genetic modification of biochemical pathways -- is changing the plant scene in three major areas:

1. growth and development control (vegetative, generative and propagation),

2. protecting plants against the ever-increasing threats of abiotic and biotic stress,

3. expanding the horizons by producing specialty foods, biochemicals and pharmaceuticals. These areas were extensively discussed at the 9th international congress of the IAPTCandB "Plant Biotechnology and In Vitro Biology in the 21st Century", held in Jerusalem in June 1998.

Growth control: Vegetative, generative and propagation

The better insight into the control of plant regeneration, morphogenesis and patterns of cell division achieved during the last two decade, is due to three major discoveries: (1) the *totipotency and regeneration ability of plant cells and tissues*, as revealed by cell culture and micropropagation, (2) the elucidation of *genes responsible for hormone production and activation* in plants, (3) active research into the mechanisms and molecular control of the *cell cycle and signal transduction pathways*, in part adopted from previous studies with animal cells, in part unique for plants. These have enabled both the *control and biotechnological manipulation* of vegetative growth, generative patterns (e.g., of flowers and seeds) and of micropropagation.

<u>Vegetative growth</u>: Morphogenetic control mechanisms are still extremely obscure, but the advent of molecular hormone and cell-cycle research is sure to lead 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/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.

Controlled cytokinin overproduction/availability can result, among other things, 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, in this respect, is the potential - as yet not practical - 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. A few of these possibilities have already been realised.

Generative development: Flowers, fruits and seeds are extremely important for agriculture. Hence, biotechnological research and development aims to interfere with and control their development and characteristics, and some of the many related studies have already produced practical applications. The major targets in *flower development* are colour, scent and senescence. Strategies for the molecular breeding of flower colour and scent include over- and underexpression of colour (anthocyanins and carotenoids) and scent (volatiles) compounds, with respect to their biosynthesis, cellular transport and targeting. Important targets for controlling *fruit development* include growth, ripening and senescence (as for vegetative growth), colour and scent (similar to flowers) and, in addition, flavour -- particularly metabolic control of sugar, acid and other flavour components.

Of great importance to fruits are biotechnological strategies for the production of seedless fruits via parthenocarpy (overproduction of auxin), pollen destruction (no fertilisation), or arrest of embryo development. The manipulation of *seed development* using biotechnological strategies is especially critical, 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, practically speaking, packages of genes that form the basis of all advanced and economically viable agricultural industries, both national and private. Biotechniques and molecular strategies are now available for the major seed-based 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: 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/or transformation stage, when only a few plants are available), and the generation of pathogen-free propagules.

This impressive application of the *principles of plant cell division and regeneration to practical plant propagation* is the result of continuous tedious studies in hundreds of laboratories worldwide, many of them in developing countries, on the standardisation of explant sources, media composition and physical state, environmental conditions and acclimatisation of in vitro plants. 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 (e.g., cuttings, tubers and bulbs, grafting).

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 utilisation of the autotrophic growth potential of cultures, and good repeatability and quality assurance of the micropropagated plants.

The need to protect: Abiotic and biotic stress tolerance

The application of molecular genetics and plant transformation to the *diagnosis and control of plant pests* has become one of the major practical success stories of plant biotechnology in the past decade. The availability of dozens of transgenic crop plants which are resistant to a range of insects, viruses and herbicides, as well as to several phytopathogenic fungi and nematodes has been validated under both field and laboratory conditions, and is of great economic importance. Moreover, applying the principles of engineering plants for resistance to these pests to other plants of agricultural importance is now considered routine, although in practice still laborious, especially for new genotypes. Apart from a wider application to additional plants, the real *challenges lying ahead* include:

1. improved expression of the target genes in the plants, especially their spatial and temporal control,

2. the use of wide-spectrum and alternative target genes to circumvent the problem of pest resistance,

3. intensified integration of biological control via the use of selected and engineered microorganisms with a biocontrol potential.

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. *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 by the local inhabitants, is often aggravated by regional climatic changes, and results in increased soil erosion and a decrease in land and agricultural productivity. It is estimated that increased salinization of arable land will have devastating global effects, resulting in 30 % land loss within the next 25 years, and up to 50 % in the year 2050. 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.

Expanding the horizons: Food, biochemicals and pharmaceuticals

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, specialised plant-derived products.

This includes two major categories of biomaterials: (1) direct improvement and modification of specialised constituents of plant origin, and (2) the manufacture in plants of non-plant compounds. Biotechniques, mostly based on the *engineering of metabolic pathways*, are now available for modifying many plant constituents that are *used in the food, chemical and energy industries*. This includes many "primary" metabolites: carbohydrates (starch synthesis, yield and allocation, production of high-amylose or high-amylopectin starch, increased sucrose synthesis for the sugar industry, fructan production, etc.), proteins (improvement of amino acid composition and protein content), oils and fats (ratio of saturated to nonsaturated fatty acids, increased content of specific valuable fatty acids like erucic acid, ricinoleic acid and others).

Many other plant constituents are either minor or non-food components, but have specific high- value applications, such as specific fatty acids as an alternative energy source, polysaccharides with heat hysteresis properties and for bioaffinity purification, temperature and salt-resistant enzymes for the food industry, etc. Moreover, the use of plants as *"bioreactors" for the production of "foreign", non-plant compounds* is gaining momentum and may eventually lead to alternative types of agriculture. This includes, for example, production of bioactive peptides, vaccines, antibodies and a range of enzymes -- mostly for the pharmaceutical industry. For the chemical industry, plants can be used to produce, e.g., polyhydroxybutyrate for the production of biodegradable thermoplastics, and cyclodextrins, which form inclusion complexes with hydrophobic substances.

Supply and demand: Where do we go from here?

Achievements today in plant biotechnology have already surpassed all previous expectations, and the future is even more promising. The full realisation and impact of the aforementioned developments depend, however, not only on continued successful and innovative research and development activities, but also on a favourable regulatory climate and public acceptance. About 12 % of the world’s land surface is used to grow crops, and the agricultural area required to support food production -- 0.44 ha / capita in 1961 -- will probably have been reduced to 0.15 ha / capita in 2050.

The intensification of agriculture with its aforementioned limitations thus requires enhanced and more efficient plant breeding and the release of economical, high-return and patentable plant-derived products. This cannot be achieved without supporting advanced research and development in biochemistry, physiology, genomics and biotechnology of agricultural plants. Plant scientists now have a central role in society, not unlike their place 300 years ago when Jonathan Swift (1667-1745) stated: "Whoever could make two ears of corn or two blades of grass to grow upon a spot of ground where only one grew before, would deserve better of mankind, and do more essential service to his country, than the whole race of politicians put together."

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