India: Biotechnology Research and Development

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Biotechnology has transformed many parts of the chemical industry, agriculture, and medicine. This area of science has little demarcation between basic and applied research, and new discoveries and innovations, in most cases, can find direct application. Innovations, techniques, and tools that have emerged and revolutionized modern biotechnology include genetic engineering, cell fusion technology, bioprocess technologies, and structure-based molecular designs including drug development, drug targeting, and drug delivery systems.

In the 1980s the Government of India considered the need for creating a separate institutional framework to strengthen biology and biotechnology research in the country. Scientific agencies supporting research in modern biology included: Council of Scientific and Industrial Research (CSIR), Indian Council of Agricultural Research (ICAR), Indian Council of Medical Research (ICMR), Department of Science and Technology, and University Grants Commission. Biotechnology was given an important boost in 1982 with the establishment of the National Biotechnology Board. Its priorities were human resource development, creation of infrastructure facilities, and supporting research and development (R&D) in specific areas. The success and impact of the National Biotechnology Board prompted the Government to establish a separate Department of Biotechnology (DBT) in February 1986. There have been major accomplishments in areas of basic research in agriculture, health, environment, human resource development, industry, safety, and ethical issues.

Basic Research

Basic research is essential on all aspects of modern biology including development of the tools to identify, isolate, and manipulate the individual genes that govern the specific characters in plants, animals, and microorganisms. Recombinant DNA (rDNA) technology is the basis for these new developments. The creativity of the scientists and the basic curiosity-driven research will be the keys to future success. India led through the work of G.N. Ramachandran, in which he elucidated the triple helical structure of collagen. The Ramachandran plot has proven to be fundamental in solving the protein structure. Areas of biosystematics using molecular approaches, mathematical modeling, and genetics including genome sequencing for human beings, animals, and plants, will continue to have priority as we move into the next century. The tremendous impact of genome sequencing is increasingly evident in many fields. As an increasing number of new genes are discovered, short, unique, expressed sequenced tags segments are used as signatures for gene identification. The power of high throughput sequencing, together with rapidly accumulating sequenced data, are opening new avenues in biosciences.

In the plant genome area, the sequencing of *Arabidopsis* and rice genome will soon be completed and cataloging and mapping of all the genes will be done.

There have been major achievements in basic bioscience in the last decade or so in India, where we have expertise in practically all areas of mod-

ern biology. The institutions under the CSIR, ICMR, ICAR, DST, and DBT have established a large number of facilities where most advanced research work in biosciences is being done. In the identification of new genes, development of new drug delivery systems, diagnostics, recombinant vaccines, computational biology, and many other related areas, considerable success has been achieved. Breakthroughs include studies on the three-dimensional structure of a novel amino acid, a long protein of mosquito (University of Poona), and demonstration of the potential of the reconstituted Sendai viral envelops containing only the F protein of the virus, as an efficient and site-specific vehicle for the delivery of reporter genes into hepatocytes (Delhi University).

Agriculture and Allied Areas

The post Green Revolution era is almost merging with the gene revolution for improving crop productivity and quality. The exploitation of heterosis vigor and development of new hybrids including apomixis, genes for abiotic and biotic resistance, and developing planting material with desirable traits and genetic enhancement of all important crops will dominate the research agenda in the next century. Integrated nutrient management and development of new biofertilizers and biopesticides would be important from the view- point of sustainable agriculture, soil fertility, and a clean environment. Stress biology, marker-assisted breeding programs, and studying the important genes will continue as priorities. We will have to switch to organic farming practices, with greater use of biological software on a large scale.

In India we have achieved the cloning and sequencing of at least six genes, developed regeneration protocols for citrus, coffee, mangrove species, and new types of biofertilizer and biopesticide formulations, including mycorrhizal fertilizers. Research to develop new genetically improved (transgenic) plants for brassicas, mung bean, cotton, and potato is well advanced. Industries have also shown a keen interest in the options of biotechnology and are participating in field trials and pilot level productions. The successful tissue culture pilot plants in the country, one at TERI in New Delhi and the other at NCL in Pune are now functioning as Micropropagation Technology Parks. This has given a new direction to the plant tissue culture industry. The micropropagation parks serve as a platform for effective transfer of technology to entrepreneurs, including training and the demonstration of technology for mass multiplication of horticulture and trees. Considerable progress has been made with cardamom and vanilla, both important crops. Yield of cardamom has increased 40 percent using tissue-cultured plants.

Between 1996 and 1998, in just eight countries, the area covered by new genetically improved transgenic plants (from 16.8 to 27.8 million hectares) (James 1998). Some of the main crops grown are soybean, corn, canola, cotton, and potato. The United States, Argentina, Brazil, and China have moved ahead quickly. The new plants exhibited herbicide, insect, and viral resistance, and overall improvement in product quality.

While the Green Revolution gave us self-reliance in food, the livestock population has provided a "White Revolution," with 80 percent of the milk in India coming from small and marginal farms. This has had a major social impact. A diverse infrastructure has been established to help farmers in the application of embryo transfer technology. The world's first IVF buffalo calf (PRATHAM) was born through embryo transfer technology at the National Dairy Research Institute, Karnal. Multiple ovulation and embryo transfer, in vitro embryo production, embryo sexing, vaccines and diagnostic kits for animal health have also been developed. Waste recycling technologies that are cost effective and environmentally safe, are being generated. The animal science area is also opening up many avenues for employment generation.

With a coastline of more than 8,000 kilometers, and two island territories of Andaman and Nicobar and Lakshadweep, there is great potential for marine resource development and aquaculture. To achieve an annual target production of 10 million metric tons of fish, scientific aquaculture offers great possibilities. In fact, aquaculture products are among the fastest moving commodities in the world. We have to continuously improve seed production, feed, health products, cryopreservation, genetic studies, and related environmental factors. This is an area

which will help substantially in the diversification of the breadbasket, and in combating nutritional deficiency.

Food Security

Food security is another area in which biotechnology offers major inputs for healthier and more nutritious food. Millions of people are malnourished, and Vitamin A deficiency affects 40 million children. There are also serious deficiencies of iodine, iron, and other nutrients. A recent UNICEF report on food and nutrition deficiencies in children describes this as a "silent, invisible emergency with no outward sign of a problem." Every year over 6 million children under the age of 5 die worldwide. About 2.7 million of these children die in India. More than half of these deaths result from inadequate nutrition.

With the advent of gene transfer technology and its use in crops, we hope to achieve higher productivity and better quality, including improved nutrition and storage properties. We also hope to ensure adaptation of plants to specific environmental conditions, to increase plant tolerance to stress conditions, to increase pest and disease resistance, and to achieve higher prices in the marketplace. Genetically improved foods will have to be developed under adequate regulatory processes, with full public understanding. We should ensure the safety and proper labeling of the genetically improved foods, so consumers will have a choice.

It is scientifically well established that an environmentally benign way of ensuring food security is through bioengineering of crops. For the 4.6 billion people in developing countries, one billion do not get enough to eat and live in poverty. Is there any other strategy or alternative? Biotechnology will provide the new tools to breeders to enhance plant capacity. Since we know that 12 percent of the world land is under agricultural crops, it is projected that the per capita availability may be reduced from 2.06 hectares to 0.15 hectare by 2050.

Plant Biotechnology

With more than 47,000 species of plants and two hot-spots of biodiversity, 8 percent of the total biodiversity of the earth is available in the Indian subcontinent. The bioresource and biodiversity constitute the mainstay of the economy of the poor people, and special emphasis is required for plant biotechnology research. Isolation of genes for abundant proteins, combining molecular genetics and chromosome maps, and a much better understanding of the evolutionary relationship of the members of the plant kingdom, have led to the potential of plant species being the major source of food, feed, fiber, medicine, and industrial raw material. Molecular fingerprinting and areas of genomics and proteonics will penetrate the barriers of fertilization to allow transfer of important characters from one plant to another. By identifying appropriate determinants of male sterility, we can extend the benefit of hybrid seeds to more crops. We must help the farmer by ensuring hybrid vigor generation after generation. Additional research on apomixis would open up such possibilities.

We have set up a National Plant Genome Research Centre at Jawaharlal Nehru University. A number of centers for plant molecular biology in different parts of the country were initially responsible for training significant numbers in crop biotechnology. There are innumerable possibilities of producing more proteins, vitamins, pharmaceuticals, coloring material, bioreactors, production of edible vaccines, therapeutic antibodies and drugs. Promising leads are available in these areas, and a number of genetically improved crops are ready for field trials of transgenic plants. Work on developing transgenic cotton, brassica, mung bean, and potato has significantly advanced.

Environment

A special area of global concern amongst the scientific community is environmental protection and conservation, and the need for a policy of sustainable development in harmony with the environment. The Stockholm Conference in 1972, and the UNCED Conference in Rio de Janeiro in 1992, both focused world attention on areas of pollution, biodiversity conservation, and sustainable development. Plants and microbes are becoming important factors in pollution control. World Bank estimates show that pollution in In-

dia is costing almost US$80 billion, as well as the human cost in terms of sickness and death. New developments such as bioindicators, phytoremediation methods, bioleaching, development of biosensors, and identification and isolation of microbial consortia are priority research areas. Significant work has been done in India, but developing a more biologically oriented approach towards pollution control would be extremely important. Cleaning up the large river systems and ensuring the destruction of pesticide residue in large slums in the city are priorities in which a biotechnological approach would be environmentally safe.

Phytoremediation to remove the high levels of explosives found in the soil has become a reality. Although it was known that some microbes can denitrify the nitrate explosives in the laboratory, they could not thrive on site. French and others (1999) have transferred this degradative ability from the microbe to tobacco plants, and these have produced a microbial enzyme capable of removing the nitrates.

Biodiversity

The global biosphere can survive only if resource utilization is about 1 percent and not 10 percent. The global environment is regulated by climate changes and biosphere dynamics. Knowledge about biodiversity accumulated in the last 250 years is being used by scientists throughout the world. There are many gene banks, botanical gardens, and herbaria for conservation purposes. There are also molecular approaches including DNA fingerprinting for plant conservation. The totality of gene species and ecosystems has become exceedingly important, not only to understand the global environment but also from the viewpoint of the enormous commercial significance of the biodiversity.

Biotechnology is becoming a major tool in conservation biology. Twelve percent of the vascular plants are threatened with extinction. Over 5,000 animal species are threatened worldwide, including 563 Indian species. India also has about 2000 species of vascular plants that are threatened.

Biodiversity is under threat, and understanding the scale of this destruction and extinction is essential. Questions such as who owns the biodiversity, who should benefit from it, and what is the role of society and the individual are pertinent. There is a Kashmiri proverb that says: *We have not inherited the world from our forefathers, we have borrowed it from our children.*

More research is needed on forests, marine resources, bioremediation methods, restoration ecology, and large-scale tree plantations. The last has reached 180 million hectares and may increase substantially in the next decade. Marine resources provide many goods and benefits including bioactive materials, drugs, and food items and must be characterized and conserved.

Medical Biotechnology

A major responsibility of biotechnologists in the 21st century will be to develop low-cost, affordable, efficient, and easily accessed health care systems. Advances in molecular biology, immunology, reproductive medicine, genetics, and genetic engineering have revolutionized our understanding of health and diseases and may lead to an era of predictive medicine. Genetic engineering promises to treat a number of monogenetic disorders, and unravel the mystery of polygenetic disorders, with the help of research on genetically improved animals. Globally, there are about 35–40 biotechnology-derived therapeutics and vaccines in use and more than 500 drugs and vaccines in different stages of clinical trials.

Every year about 12 million people die of infectious diseases. The main killers according to WHO are acute respiratory infection, diarrheal diseases, tuberculosis, malaria, hepatitis, and HIV-AIDS. There are vaccines being developed for many diseases, and diagnostic kits for HIV, pregnancy detection, and hepatitis are being developed. The technologies have been transferred to industry.

The Department of Biotechnology has developed guidelines for clinical trials for recombinant products, which have now been accepted by the Health Ministry and circulated widely to industry. Promising leads now exist to develop vaccines for rabies, *Mycobacterium* tuberculosis, cholera, JEV, and other diseases. Recombinant hepatitis B vaccine and LEPROVAC are already on the market. There is a Jai Vigyan technology mission on the development of vaccines and diagnostics. A

National Brain Research Centre is being established to improve knowledge of the human brain and the brain diseases.

The discovery of new drugs and the development of the drug delivery system are increasingly important. Bioprospecting for important molecules and genes for new drugs has begun as a multi-institutional effort. A recombinant vaccine for BCG and hepatitis is being developed. The age-old system of Ayurveda practiced in India needs to be popularized and made an integral part of health care. The global market for herbal products may be around US$5 trillion by 2050.

Industrial Biotechnology

Advances in biotechnology can be converted into products, processes, and technologies by creating an interdisciplinary team. The pharmaceutical sector has had a major impact in this field, as rare therapeutic molecules in the pure form become available. Diagnostics have expanded, with over 600 biotechnology-based diagnostics (valued at about US$20 billion worldwide) now available in clinical practice. The polymerase chain reaction (PCR)-based diagnostics are the most common. Indian efforts in the diagnostic area have been commendable, and it is expected that sales will rise from about US$235 million to US$470 million in the next century.

The consumption of biotechnology products is expected to increase from US$6.4 billion to about US$13 billion by 2000. Industrial enzymes have emerged as a major vehicle for improving product quality. In India a number of groups are gearing up to produce industrial enzymes such as alpha-amylose, proteases, and lipases, increasing three-fold by the end of the century, which will match or surpass the computer industry in size, importance, and growth. India is now producing 13 antibiotics by fermentation. Capacity exists to produce important vaccines such as DPT, BCG, JEV, cholera, and typhoid. Cell culture vaccines such as MMR and rabies, and hepatitis-B, have also been introduced

Bioinformatics

The coming together of biotechnology and informatics is paying rich dividends. Genome projects, drug design, and molecular taxonomy are all becoming increasingly dependent on information technology. Information on nucleotides and protein sequences is accumulating rapidly. The number of genes characterized from a variety of organisms and the number of evolved protein structures are doubling every two years. DBT has established a national Bioinformatics Network with ten Distributed Information Centres (DICs) and 35 sub-DICs. A Jai Vigyan Mission on establishment of genomic databases has been started, with a number of graphic facilities created throughout the country. This system has helped scientists involved in biotechnology research.

Ethical and Biosafety Issues

The bioethics committee of UNESCO established in 1993 has evolved guidelines for ethical issues associated with the use of modern biotechnology.

Biosafety guidelines for genetically improved organisms (GIOs) need to be strictly followed to prevent harm to human health or the environment. A three-tier mechanism of Institutional Biosafety Committees has been instituted in India: the Review Committee on Genetic Manipulation, the Genetic Engineering Approval Committee, and the state level coordination committees. It is important to give a clear explanation of the new biotechnologies to the public to allay their fears. New models of cooperation and partnership have to be established to ensure close linkages among research scientists, extension workers, industry, the farming community, and consumers.

Gene transformation is done worldwide with four broad objectives: (a) to develop products with new characteristics; (b) to develop pest and disease resistance; (c) to improve nutritional value; and (d) to modify fruit ripening to obtain longer shelf life. Thus the aims and objectives are laudable and the tools are available. The new technology does, however, call for a cautious approach following appropriate biosafety guidelines.

About 25,000 field trials of genetically modifed crops have been conducted worldwide. The anticipated benefits are better planting material, savings on inputs, and genes of different variet-

ies can be introduced in the gene pool of crop species for their improvement. The potential risks include weediness, transgene flow to nontarget plants, and the possibility of new viruses developing with wider host range and their effects on unprotected species. For crops such as corn and cotton with single gene introductions, there is very little problem expected. When multiple genes are involved scientists have to be more cautious.

The time has arrived for a serious look at ethical and biosafety aspects of biotechnology. Researchers, policymakers, NGOs, progressive farmers, industrialists, government representatives, and all concerned players need to come together and share a platform to address the following issues.

- Environmental safety
- Food and nutrition security
- Social and economic benefits
- Ethical and moral issues
- Regulatory issues.

Human Resource Development

There are about 50 approved MS, postdoctoral, and MD training programs in biotechnology in progress or just about to start, in different institutions and universities covering most Indian States. Short-term training programs, technician training courses, fellowships for students to go abroad, training courses in Indian institutions, popular lecture series, awards, and incentives form an integral part of the human resource development activities in India. A special feature of the program has been that since 1996 many students after completion of their training course join industries or work in biotechnology-based programs in institutions and laboratories. National Bioscience Career Development Awards have been instituted. Special awards for women scientists and scholarships to the best students in biology help promote biotechnology in India and give recognition and reward to the scientists.

Some Special Programs

Biotechnology-based activities to benefit the poor and weaker sections and programs for women have been launched. A unique feature is the establishment of a Biotechnology Golden Jubilee Park for Women which will encourage a number of women entrepreneurs to take up biotechnology enterprises that benefit women in particular. This will also encourage women biotechnologists to develop relevant technologies.

States are taking a keen interest in developing biotechnology-based activities. The States of Uttar Pradesh, Arunachal Pradesh, Madhya Pradesh, Kerala, West Bengal, Jammu and Kashmir, Haryana, Mizoram, Punjab, Gujarat, Meghalaya, Sikkim and Bihar have already started large-scale demonstration activities and training programs.

Investment Required

The Indian Government has made substantial investments in biotechnology research. Bringing Indian biotechnology products to market will require the involvement of large and small entrepreneurs and business houses. This will require substantial investments from Indian and overseas investors. The worldwide trend is that large companies are becoming major players in development of biotechnology products, and also in supporting product-related biotechnology research.

Expectations

In the years ahead, biotechnology R&D should produce a large number of new genetically improved plant varieties in India, including cotton, rice, brassicas, pigeonpea, mung bean, and wheat. Tissue culture regeneration protocols for important species such as mango, saffron, citrus, and neem will lead to major commercial activities. Micropropagation technology will provide high-quality planting materials to farmers. Environment-friendly biocontrol agents and biofertilizer packages will hopefully be made available to farmers in such a way that they can produce these in their own fields. The country should be in a position to fully utilize, on a sustainable basis, medicinal and aromatic plants. The development through molecular biology of new diagnostic kits and vaccines for major diseases would make the health care system more efficient and cheaper. Genetic counselling clinics, molecular probes, and fingerprinting techniques should all

be used to solve the genetic disorders in the population. The establishment of ex situ gene banks to conserve valuable germplasm and diversity, and a large number of repositories, referral centers for animals, plants, and microorganisms should be possible. Detailed genetic readouts of individuals could be available. Information technology and biotechnology together should become a major economic force. It is expected that plants as bioreactors would be able to produce large numbers of proteins of therapeutic value, and many other important items. The recent discovery of the gene for recalcitrant species was a landmark event. In vitro mass propagation can be carried out on any desired species with nonrandom programming. Certainly the 21st century could witness a major increase in new bioproducts generated through modern biology.

To achieve the goal of self-reliance in this field, India will require a strong educational and scientific base, clear public understanding of the value of new biotechnologies, and involvement of society in many of these biological ventures. India has a large research and educational infrastructure comprising 29 agriculture universities, 204 central and state universities, and more than 500 national laboratories and research institutions. It should therefore be possible to develop capabilities and programs so that these institutions act as regional hubs for the farming community, where they can get direct feedback about new technological interventions. It will be equally important to establish strong partnerships and linkages with industry, from the time a research lead has emerged until the packaging of the technology and commercialization are achieved. Arther Kornberg, Nobel Laureate, stated: "*Much has been said about the future impact of biotechnology on industrial development, but this does not yet apply to the less developed countries that lack this infrastructure and industrial strength. In view of the current power of biotechnology and its even brighter future, there is no question that the less developed countries must now position and strengthen their status in biotechnology.*"

Kornberg further stressed that :"*What a tragedy it would be if these enlarged concepts of genetics, biology and chemistry were available only to a small fraction of the world population located in a few major centres of highly developed countries.*"

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