

Agricultural biotechnology research in India: Status and policies

Manju Sharma*, K. S. Charak and T. V. Ramanaih

Department of Biotechnology, Block-2, CGO Complex, New Delhi 110 003, India

Agriculture is a way of life for more than sixty per cent of India's population. The cultivation of land not only sustains their livelihood but also provides a social milieu for their day-to-day living. No wonder the hopes, despairs, joys and sorrows of rural communities are woven around what the land provides. Around 35 years ago, agricultural production in India got a major boost with the introduction of dwarf varieties of wheat and rice. The introduction of these varieties led to a dramatic increase in the yields of the two crops. Some productivity enhancement came through the use of hybrids in corn, sorghum and millet although the area under cultivation of these crops has steadily decreased in the last decade. In the last 10 years the yields of rice and wheat have also plateaued out. The productive agricultural areas in the North, due to continuous rice-wheat cultivation are encountering serious problems of sub-soil water depletion, deficiency of micronutrients in the soil and increase in the use of pesticides, fungicides and herbicides to control pests, pathogens and persistent weeds. Agricultural production is becoming more and more dependent on agrochemicals, thereby increasing input costs and causing significant damage to the environment and human health. Both farmers and consumers are at the receiving end – farmers by exposure to agrochemicals and consumers due to residues of agrochemicals in the consumed food.

While there is self-sufficiency in cereal grains at present, the yields and productivity of dryland crops, mostly grain legumes and oilseeds remain low and no major breakthroughs in productivity enhancement and yield stabilization have been achieved. Currently, India is importing both grain legumes and edible oils to meet internal demands. India's population is expected to reach approximately 1.5 billion by 2050. It is estimated that around 300 million (roughly 30%) of India's population is suffering from malnutrition. Due to socio-economic reasons, women and children are more at the receiving end in terms of micronutrient deficiency and low calorie intake. Nutritional security for everyone would require more extensive availability of grain legumes, edible oils, fruits and vegetables, milk and poultry products. The challenges of malnutrition, enhanced productivity and crop diversification can be met by better resource man-

agement and by breeding more productive, more nutritious and at the same time less resource input demanding crops.

Crop biotechnology, which broadly includes areas of development of transgenic crops, structural and functional genomics and marker-assisted breeding could provide us with the vital breakthroughs to achieve improvements in both quality and quantity in a sustainable manner. With the advent of techniques of genetic engineering in the early seventies, the natural barrier to gene exchange has been removed. Sequences from varied sources like bacteria, viruses and eukaryotic systems can be transferred to plants to develop transgenic crop varieties. Achievements, to date, in plant biotechnology have surpassed all previous expectations and with the development of high throughput instruments, the future is even more promising.

In this article we outline the contributions made by the Department of Biotechnology in promoting research and development in the area of crop biotechnology and in establishing proper regulatory regimes for transgenic crops.

Agriculture biotechnology research in India

Recognizing the importance and potential of biotechnology, the Government of India set up the National Biotechnology Board in 1982 which became a full-fledged department (called as Department of Biotechnology, DBT) in 1986 under the Ministry of Science and Technology. The plan-wise budget of DBT from 1992 onwards in the area of crop biotechnology is given in Table 1. To provide research and human resource development in crop biotechnology, DBT supported the establishment of Centres for Plant Molecular Biology (CPMB) in 1990. A total of six such centres were set up initially at various Universities/Institutions namely, Jawaharlal Nehru Univer-

Table 1. Plan expenditure by DBT in crop biotechnology. Amount (Rs in lakhs)

| Scheme | 8th Plan | 9th Plan | 10th Plan | |
|--------------------|-----------------------|-----------------------|----------------|----------------|
| | 1992–1997 (Actual) | 1997–2002 (Actual) | 2002– 2007* | 2002– 2003* |
| Crop biotechnology | 1916.00 | 2897.00 | 7500.00 | 1400.00 |

*Budget estimates.

*For correspondence. (e-mail: manju@dbt.nic.in)

sity (New Delhi), Madurai Kamaraj University (Madurai), Tamil Nadu Agricultural University (Coimbatore), Osmania University (Hyderabad), National Botanical Research Institute (Lucknow) and Bose Institute (Kolkata). A seventh centre was established at the University of Delhi South Campus in 1997. A total financial outlay of Rs 45.98 crores was made available for the development and research activities of CPMBs.

Most of the research on crop biotechnology currently underway in India is being supported by the Government and the DBT has been the main agency amongst government departments to support research endeavours in this area. The DBT has supported over the last 12 years a large number of research projects which deal with the development of *in vitro* regeneration and genetic transformation protocols of important crop species grown in India, the development of transgenics with genes of agronomic importance and several projects dealing with basic research in plant molecular biology.

To bring investigators with expertise in different facets of biology together to address defined research goals, a number of multi-institutional projects have been launched which include development of transgenics for resistance to geminiviruses in cotton, mungbean and tomato, resistance to rice tungro disease, resistance to bollworms in cotton, development of nutritionally improved potato with a balanced amino acid composition using a heterologous protein from *Amaranthus* and development of molecular methods for heterosis breeding. Another set of projects include tagging of quality traits in rice and mustard, resistance to karnal bunt and rust in wheat and an extensive research network on rice biotechnology, which includes work on genomics, mapping, improvement in nutritional quality and resistance to biotic and abiotic stresses. Another multi-institutional project on tagging of quality traits in wheat by molecular markers has given some interesting leads. A number of markers linked to grain protein content, seed size, bread-making quality, etc. have been identified.

Transgenics being developed in the country both by the public and the private sector are listed in Tables 2 and 3.

To further strengthen research in the area of crop biotechnology, a new institute, National Centre for Plant Genome Research (NCPGR) has been established in New Delhi with a mandate to strengthen plant biotechnology research in India.

Emphasis on genomics

Rice production in India holds the key to the food security of the country. After detailed consultations with experts at the National and International level, DBT and the Indian Council of Agricultural Research (ICAR) jointly took the initiative to participate in the international rice genome-sequencing programme (IRGSP) in June, 2000. Two laboratories in India, one at the

University of Delhi South Campus and the other at the Indian Agricultural Research Institute (IARI) in New Delhi are jointly sequencing part of chromosome II (region between 57.3 cM and 110.1 cM of the genetic map). This region carries genes for a number of biotic and abiotic stresses and genes encoding some agronomically important traits. Around 15.58 Mb of DNA has already been sequenced till December 2002. A high quality draft sequence of rice genome by IRGSP has been declared completed by the Japanese Prime Minister Junichiro Koizumi on 18 December 2002 in Tokyo.

In order to take advantage of sequence information in rice, a workshop was organized in May 2002 to identify areas which should be funded for functional genomics of rice. It was decided to develop research programmes in the areas of:

- (i) salinity and drought stress,
- (ii) biotic stresses,
- (iii) mapping of loci contributing to high yield.

The project on genome sequencing will also allow rapid progress in the characterization of allelic diversity available in *Oryza sativa* and related species and genera for resistance to diseases and pests of rice.

Future developments

In the 10th Five Year Plan (2002–2007) the following areas will be given high priority:

- Rice genomics with particular emphasis on the study of genetic variability for achieving both yield increase and stabilization in the crop.
- Molecular maps in rice, wheat and grain legume crops. To date, a molecular map with reasonable density (around 1000 AFLP markers) has been developed only for mustard (*Brassica juncea*). In wheat, tagging of some interesting traits has been accomplished. The work on cereal crops will continue and mapping work will be initiated in grain legume crops. Molecular maps will be used for mapping of QTLs for abiotic stresses, plant architecture, and yield and mapping of loci conferring resistance to pests and pathogens.
- Transgenics developed in various crops through DBT funding will be tested under appropriate containment conditions and if found useful will be subjected to nutritional and toxicological tests to determine their suitability for cultivation in the farmer's field.
- Research on development of pollination control methods for the production of hybrid seed will be further strengthened. Fixation of heterosis through apomixis will be another area of research.
- Particular emphasis will be given on an improvement in the nutritional status of some of our major crops. To begin with, the three target crops will be rice, wheat and potato. High-yielding rice varieties grown in India will be either transformed with constructs that would

Table 2. Major Indian developments in transgenic research and application in public sector

| Institute | Plants/crops | Transgene(s) inserted | Aim of the project |
|---|-------------------|--|---|
| AAU, Jorhat, Assam | Chickpea | Bean <i>alpha AI</i> | To generate plants resistant to bruchids |
| Bose Institute, Kolkata | Rice | <i>S-adenosylmethionine decarboxylase</i> | To generate plants tolerant to stress |
| Central Institute for Cotton Research, Nagpur | Cotton | <i>Bt. cry</i> gene(s) | To generate plants resistant to lepidopteran pests |
| Central Potato Research Institute, Simla | Potato | <i>Bt. cryIA(b)</i> | To generate plants resistant to lepidopteran pests |
| Central Tobacco Research Institute, Rajahmundry | Tobacco | <i>Bt. cryIA(b)</i> and <i>cryIC</i> | To generate plants resistant to <i>Helicoverpa armigera</i> and <i>Spodoptera litura</i> |
| Centre for Cellular and Molecular Biology, Hyderabad | Rice | <i>bar</i> | To generate herbicide-tolerant plants |
| Central Rice Research Institute, Cuttack | Rice | <i>Bt cryIA(b)</i> <i>Xa21</i> | To develop plants resistant to lepidopteran pests, bacterial blight/disease |
| Delhi University, South Campus, New Delhi | Mustard/rapeseed | <i>bar</i> , <i>barnase</i> , <i>barstar</i> | To generate herbicide-tolerant plants, male-sterile and restorer lines for hybrid seed production |
| | Rice | <i>Pyruvate decarboxylase</i> and <i>alcohol dehydrogenase</i> | To generate plants tolerant to flooding |
| | Tomato | <i>Ctx-B</i> and <i>Tcp</i> antigens of <i>Vibrio cholerae</i> | Edible vaccine development |
| | Brinjal | Chitinase, glucanase and thaumatin encoding genes | To generate plants resistant to diseases |
| | Wheat | <i>bar</i> , <i>HVA1</i> , <i>PIN2</i> | Resistance against biotic and abiotic stresses |
| | Rice Pusa basmati | <i>codA</i> , <i>cor47</i> , | Resistance against biotic and abiotic stresses |
| Directorate of Rice Research, Hyderabad | Rice | <i>Xa-21</i> , <i>cryIA(b)</i> | To generate plants resistant to lepidopteran pests and bacterial and fungal diseases |
| Indian Agricultural Research Institute, New Delhi | Brinjal | <i>Bt. cryIA(b)</i> | To generate plants resistant to lepidopteran pests |
| | Tomato | <i>Bt. cryIA(b)</i> | To generate plants resistant to lepidopteran pests |
| | Cauliflower | <i>Bt. cryIA(b)</i> | To generate plants resistant to <i>Plutella scylostella</i> |
| | Cabbage | <i>Bt. cryIA(b)</i> | To generate plants resistant to <i>P. scylostella</i> |
| | Rice | <i>Bt cryIA(b)</i> , <i>chitinase</i> | To generate plants resistant to lepidopteran pests |
| | Mustard/rapeseed | <i>Arabidopsis annexin</i> gene | To generate stress-tolerant plants |
| | Mustard/rapeseed | <i>Choline dehydrogenase</i> | To generate abiotic stress-tolerant plants |
| | Potato | ACC synthase | To control fruit ripening |
| | Tomato | ACC synthase | To control fruit ripening |
| | Banana | ACC synthase | To control fruit ripening |
| | Tobacco | Chitinase, glucanase and <i>RIP</i> | To generate plants resistant to fungal attack |
| | <i>Brassica</i> | Chitinase, glucanase and <i>RIP</i> | To generate plants resistant to fungal attack |
| | Pigeonpea | <i>Protease inhibitor</i> and <i>lectin</i> genes | To generate plants resistant to bollworms and aphids |
| IARI sub-station, Shillong | Rice | <i>Bt. cryIA(b)</i> | To generate plants resistant to yellow stem borer |
| International Centre for Genetic Engineering and Biotechnology, New Delhi | Tobacco | <i>Bt. cryIIa5</i> | To generate plants resistant to <i>Spodoptera litura</i> |
| | Rice | <i>Gm2</i> | To generate plants resistant to gall midge |
| International Crop Research Institute for Semi-Arid Tropics, Hyderabad | Chickpea | <i>PGIP</i> | To generate plants resistant to fungal pathogens |

(Table 2. Contd)

SPECIAL SECTION: TRANSGENIC CROPS

| Institute | Plants/crops | Transgene(s) inserted | Aim of the project |
|---|----------------------|--|---|
| Indian Institute of Horticultural Research, Bangalore | Muskmelon | <i>Rabies glycoprotein</i> gene | To develop edible vaccines |
| | Tomato | <i>Leaf curl virus</i> sequence | To generate plants resistant to leaf curl virus |
| | Tomato | Chitinase and glucanase | To generate plants resistant to fungal diseases |
| Jawaharlal Nehru University, New Delhi | Potato | <i>Ama-1</i> * | To generate nutritionally enriched plants |
| | Tomato | <i>OXDC</i> * | To generate plants resistant to fungal infection |
| Madurai Kamaraj University, Madurai | Blackgram | Coat protein and replicase genes of <i>Vigna mungo</i> yellow mosaic virus | To develop viral-resistant plants |
| | Blackgram | <i>Dianthin</i> and <i>barnase</i> gene or <i>bar</i> | Development of insect resistant and herbicide-tolerant plants |
| | Rice | Chitinase, β -1, 3- <i>glucanase</i> and <i>osmotin</i> genes | To develop plants resistant to fungal infection |
| | Coffee | Chitinase, β -1, 3- <i>glucanase</i> and <i>osmotin</i> genes | To develop plants resistant to fungal infection |
| Narendra Dev University of Agriculture, Faizabad | Rice | <i>CryIA(b)</i> gene | To generate plants resistant to lepidopteran pests, bacterial and fungal diseases |
| National Botanical Research Institute, Lucknow | Cotton | <i>CryIE</i> and <i>CryIC</i> with terminal altered at C-end | To develop transgenic resistant to <i>Spodoptera litura</i> and <i>Heliothis armigera</i> |
| Punjab Agricultural University, Ludhiana | Rice Pusa basmati | <i>CryIAb</i> , <i>CryIAc</i> | For resistance against yellow stem borer |
| Tata Energy Research Institute, New Delhi | Mustard | <i>Ssu-maize Psy</i> and <i>Ssu-tpCrtI</i> gene | To generate plants containing high levels of β -carotene |
| Tamil Nadu Agricultural University, Coimbatore | Rice | <i>GNA</i> gene | To generate plants resistant to pests gall midge |
| University of Agricultural Sciences, Bangalore | Muskmelon | <i>Rabies glycoprotein</i> gene | To develop edible vaccines |

*Genes isolated in India. In most of the viral work, sequences for pest-derived resistance (PDR) have been isolated from the viral strains predominant in India.

code for high β carotene content or by back-crossing these varieties to 'Golden rice' transgenics already developed in Switzerland. Improving the iron content in rice grains will be another thrust area. In potato, nutritionally enhanced transgenic Indian varieties of potato carrying the *amal* gene are already under advanced field trials. If found suitable, these will be released for cultivation.

- Transgenic approaches to senescence retardation in fruits will be a major thrust area. It is important to reduce post-harvest losses in fruits and vegetables as under tropical and sub-tropical conditions senescence is much more rapid and energy costs of cold-storage are very high.

Biosafety considerations

Safety of transgenic material is a major concern. A major contribution of DBT has been to set up, in collaboration with other government departments, a framework for the

evaluation and eventual clearance of transgenic crops for field cultivation. The release of transgenic crops is governed by the Indian Environment Protection Act (EPA)–1986 which came into force from May 1986. The act provides a framework for the protection and improvement of environment. Later, the rules and regulations for the manufacture, use, import, export and storage of hazardous microorganisms, genetically engineered organisms or cells were notified under EPA 1986 on 5 December 1989.

A mechanism based on interaction between committees and different departments of Government of India has been set up (Figure 1). Such materials will have to meet with the approval of the following Committees: Institutional Biosafety Committee (IBSC), Review Committee on Genetic Manipulation (RCGM) and Genetic Engineering Approval Committee (GEAC).

Under the EPA 1986, the GEAC examines from the viewpoint of environmental safety and issues clearance

Table 3. Transgenic lines in advanced stage of development for field trials in private sector

| | | | | |
|---------------------------------------|----------------------------------|--|--|--|
| Ankur Seeds Ltd, Nagpur | Cotton* | <i>Cry1A(c)</i> | To generate plants resistant to lepidopteran pests | |
| Hybrid Rice International, Gurgaon | Rice | <i>Cry1A(b)</i> , <i>cry9C</i> and <i>bar</i> genes | To develop plants resistant to lepidopteran pests and herbicide tolerance | |
| Indo-American Hybrid Seeds, Bangalore | Tomato | <i>Alfalpa glucanase</i> and <i>Tomato leaf curl virus</i> genes | To generate plants resistant to viral and fungal attacks | |
| MAHYCO, Mumbai | Cotton* | <i>Cry1A(c)</i> | To generate plants resistant to lepidopteran pests | |
| | Cotton* | <i>CP4 EPSPS</i> | To generate plants for resistance to herbicide glyphosate | |
| | Cotton* | <i>CryX</i> gene | To generate plants resistant to lepidopteran pests | |
| | Corn* | <i>Cry1A(b)</i> | To generate plants resistant to lepidopteran pests | |
| | Pigeonpea | <i>GUS</i> | For transformation work | |
| MAHYCO Research Foundation, Hyderabad | Rice | <i>Cry1A(c)</i> , <i>Xa21</i> and <i>GNA</i> genes | To generate plants resistant to lepidopteran pests, bacterial blight and sucking pests | |
| | Mustard | <i>CP4 EPSPS</i> | To generate plants tolerant to herbicide | |
| | Rice | <i>Bacterial blight resistance</i> conferring <i>Xa-21</i> gene | To generate plants resistant to bacterial blight | |
| | Proagro PGS (India) Ltd, Gurgaon | <i>Brassicata</i> mustard | <i>Bar</i> , <i>barnase</i> , <i>barstar</i> | To develop superior hybrid cultivars |
| | Syngenta India Ltd, Pune | Tomato* | <i>Cry1A(b)</i> | To generate plants resistant to lepidopteran pests |
| Brinjal* | | <i>Cry1A(b)</i> | To generate plants resistant to lepidopteran pests | |
| Cauliflower* | | <i>Cry1H/cry9C</i> | To generate plants resistant to lepidopteran pests | |
| Cauliflower* | | <i>Bar</i> , <i>barnase</i> , <i>barstar</i> | To develop superior hybrid cultivars | |
| Cabbage* | | <i>Cry1H/cry9C</i> | To generate plants resistant to lepidopteran pests | |
| Cotton* | | <i>Vip-3</i> gene | To generate plants resistant to lepidopteran pests | |
| Maize* | | <i>Cry1A(b)</i> | To generate plants resistant to lepidopteran pests | |

*Transgenics developed elsewhere are being backcrossed to inbred lines to develop commercially viable hybrids that can be grown in different agroclimatic regions of the country.

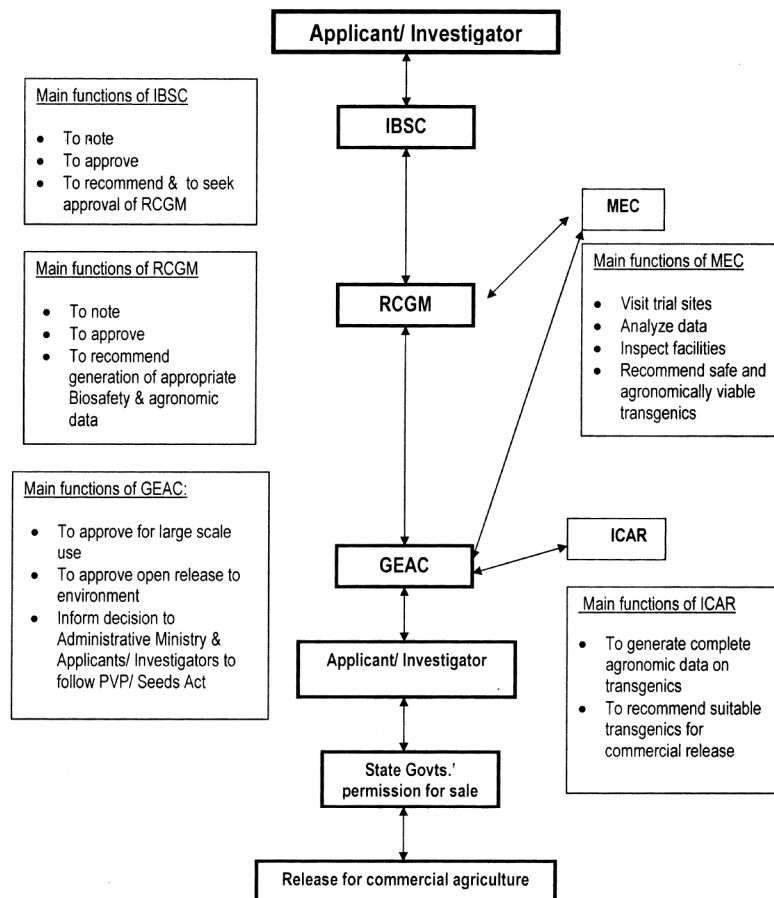


Figure 1. Procedures involved in commercialization of indigenously developed transgenic crops.

or the release of genetically engineered organisms/transgenic crops and products into the environment. The GEAC also grants permits to conduct experimental and large-scale field trials which are beyond the limit of 20 acres. In case of transgenic crops, applicants are required to seek clearance from the Ministry of Agriculture, which will accord the final clearance of transgenic crop varieties for agricultural activities (Figure 1).

Bt cotton was released for commercial cultivation in March 2002, following the biosafety procedures laid down by the Government of India. Three cotton hybrids have been granted permission for field sowing and are currently in the fields in six states namely, Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Karnataka and Tamil Nadu. Transgenic mustard hybrids are also in advanced field trials and if found suitable, may be released for general cultivation.

Conclusions

Regulatory mechanisms for the release of transgenics are in place. A Plant Variety Protection Bill (PVP) has been cleared by the Indian Parliament and will soon become operational. These developments should encourage industry to invest in the seed sector. The Government of India and the Department of Biotechnology are committed to

fund research in the area of crop biotechnology. The support from the Government for funding research in crop biotechnology has increased. If tangible products come through, given the importance of agriculture to the economy, prospects of higher financial outlays for crop biotechnology are bright.

To address the general concerns of public on genetically modified food, it is of utmost importance that the regulatory system that has been established is followed strictly. The environmental effect of transgenic crops should be assessed and compared with non-transgenic crops grown with conventional practices. The field performance and drift of transgenes, either to wild relatives of the crop or to non-transgenic varieties will have to be monitored over a considerable period of time. While introducing transgenic varieties, every effort will have to be made to maintain the conventionally bred varieties for use as germplasm for future plant breeding.

To conclude, the Department of Biotechnology in collaboration with other government agencies and the public and private seed sector wants to fully exploit the opportunities provided by crop biotechnology to advance Indian agriculture towards better quality and higher overall productivity which can be sustained for many decades to come.