Development, Production and Quality Control of GM Seeds in India

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Genetically modified crops (GMCs, GM crops, Transgenic crops or biotech crops) are plants, the DNA of which has been modified using genetic engineering techniques, to resist pests and agents causing harm to plants and to improve the quality.
Genetic engineering techniques are much more precise than mutagenesis (mutation breeding) where an organism is exposed to radiation or chemicals to create a non-specific but stable change. Other techniques by which humans modify plants include selective breeding; plant breeding, and somaclonal variation.

In most cases the aim is to introduce a new trait to the plant which does not occur naturally in this species. Examples include resistance to certain pests, diseases or environmental conditions, or the production of a certain nutrient or pharmaceutical agent.

History of genetic engineering

The first genetically modified plant had produced in 1982, using an antibiotic-resistant tobacco plant. The first field trials of genetically engineered plants occurred in France and the USA in 1986, when tobacco plants were engineered to be resistant to herbicides. In 1987, Plant Genetic Systems (Ghent, Belgium), founded by Marc Van Montagu and Jeff Schell, was the first company to develop genetically engineered (tobacco) plants with insect tolerance by expressing genes encoding for insecticidal proteins from *Bacillus thuringiensis* (Bt). The People's Republic of China was the first country to allow commercialized transgenic plants, introducing a virus-resistant tobacco in 1992. The first genetically modified crop approved for sale in the U.S., in 1994, was the *FlavrSavr* tomato, which had a longer shelf life. In 1994, the European Union approved tobacco engineered to be resistant to the herbicide bromoxynil, making it the first commercially genetically engineered crop marketed in Europe. In 1995, Bt Potato was approved safe by the Environmental Protection Agency, making it the first pesticide producing crop to be approved in the USA. The following transgenic crops also received marketing approval in the US in 1995: canola with modified oil composition (Calgene), *Bacillus thuringiensis* (Bt) corn/maize (Ciba-Geigy), cotton resistant to the herbicide bromoxynil (Calgene), Bt cotton (Monsanto), soybeans resistant to the herbicide glyphosate (Monsanto), virus-resistant squash (Asgrow), and additional delayed ripening tomatoes (DNAP, Zeneca/Peto, and Monsanto). In 2000, with the production of golden rice, scientists genetically modified food to increase its nutrient value for the first time.

GM Crops in India

In India, the government had allowed for field trials of 20 genetically modified (GM) crops in the country. Out of the 20 crops approved, field trials of only transgenic cotton, corn and mustard had been initiated after the obtainment of no-objection certificates (NOC) from respective state governments. NOCs from respective state governments for other crops are still awaited.
Bt cotton, a patented product of Monsanto, remains the first and only GM crop to be commercially cultivated in India, starting in 2002. Following Bt cotton, the Indian government tried to introduce

Bt brinjal (eggplant) towards the end of 2009. It was the first GM food crop to be commercialized in India. However, after serious concerns were raised on its safety by scientists, ecologists, farmers and consumers, an indefinite moratorium was put on it in 2010 by the Ministry of Environment and Forest.

Chronology of Development and Approval of *Bt*-Cotton in India

1995	Mahyco applied to DBT (Department of Biotechnology, Govt. of India) for permission to import a small stock of Bollgard® (*Bt* cotton) seeds from Monsanto Company, USA. DBT gave permission.
1996	A nucleus stock of 100 gms of cotton seeds of the variety Cocker 312 containing the Bollgard® *Bt* gene, *cry 1Ac,* was received by Mahyco from Monsanto, USA. Initiated crossing with the Indian cotton breeding lines to introgress *cry 1Ac* gene. 40 elite Indian parental lines were converted for *Bt* trait.
1996-1998	Risk-Assessment Studies conducted using *Bt*-cotton seeds from converted Indian lines.
1998 – 1999	Multi-location field trials at 40 locations in 9 states to assess agronomic benefits and safety. Data submitted to RCGM (Review Committee for Genetic Modification), Ministry of Science & Technology, Govt. of India.
1999 – 2000	Field trials repeated at 10 locations in 6 states. Data submitted to RCGM.
2000	July 2000 – Based on the recommendation of RCGM, the GEAC (Genetic Engineering Approval Committee), Ministry of Environment & Forests, Govt. of India, gave approval for Mahyco to conduct large scale field trials in 85 ha and also undertake seed production in 150 ha.
2001	*Kharif* 2001 – Large scale field trials covering 100 ha. Field trials were also conducted by All India Coordinated Cotton Improvement Project of the Indian Council of Agricultural Research (ICAR).
2002	On 26 March 2002, GEAC approved Mahyco's three *Bt*-cotton hybrids, viz. MECH 12, MECH 162 and MECH 184, for commercial cultivation in India. This approval was initially valid for three years and also stipulated a few conditions. This is a landmark decision as *Bt*-cotton is the first-ever transgenic crop to receive such a regulatory approval in India.

Based on the recommendation of RCGM, the Genetic Engineering Approval Committee (GEAC), in its 32nd meeting held in New Delhi on 26th March 2002, approved Mahyco's *Bt*-cotton for commercial cultivation, pronouncing it to be safe and beneficial. This is a landmark decision as *Bt*-cotton happens to be the first-ever agricultural biotech product to receive official approval and with it India made its long awaited entry into commercial agricultural biotechnology. This approval has specified three *Bt* hybrids, namely Mech 12, Mech 162 and Mech 184 which had undergone all the trials and it was initially granted for three years. The approval also stipulated certain other conditions and one of them was that every *Bt*-cotton field shall be fully surrounded by a 'refuge' crop comprising the same non-*Bt*-cotton hybrids and the size of the refuge shall be at least five rows of non-*Bt* or 20% of the total sown area whichever is greater. The idea of 'refuge' is to serve as a strategy to produce *Bt* sensitive insects thereby helping to prevent or delay the development of resistance by bollworms to the in plants produced *Bt* protein. Besides, 'refuge' can act as a 'pollen sink' area to some extent.

Bt

The Bt is a short form of ubiquitous soil bacterioum Bacillus thuringiensis. This bacterium is gram positive and spore forming that forms parasporal crystals during stationary phase of its growth cycle. The synthesized crystalline proteins called 'endotoxins' are highly toxic to certain insects. They kill the insect by acting on the epithelium tissues of midgut of caterpillars. These proteins often appear microscopically as distinctly shaped crystals and constitute about 20-30% of dry weight of sporulated cultures. These proteins are characterized by their insecticidal activity and are therefore grouped into four classes i.e. Lepidoptera-specific (Cry I), Lepidoptera and Diptera-specific (Cry II), Coleoptera-specific (Cry III) and Diptera-specific (Cry IV). Different strains of Bt produce more than 25 different but related insecticidal crystal proteins (ICPs). These are toxic to larvae of different insects including disease vectors and many agricultural pests. Cotton bollworms belong to the order Lepidoptera and therefore are sensitive to Bt Cry I and Cry II proteins, which are specific to them.

Bt Cotton

A genotype or individual which is developed by the techniques of genetic engineering is referred to as transgenic. Transgenic plants contain foreign gene or genetically modified gene of the same species. The foreign gene may be from a distantly related species, closely related species or unrelated species or from micro-organisms such as fungi, bacteria and viruses. Bt cotton refers to transgenic cotton which contains endotoxin protein inducing gene from soil bacterium *Bacillus thuringiensis*. The transgenic cotton is of two types viz.

1. Bollgard - confers resistance to bollworms and the latter is resistant to herbicides.
2. Roundup ready cotton - area under herbicide resistant transgenic cotton is restricted to USA.

Development of Bt Cotton

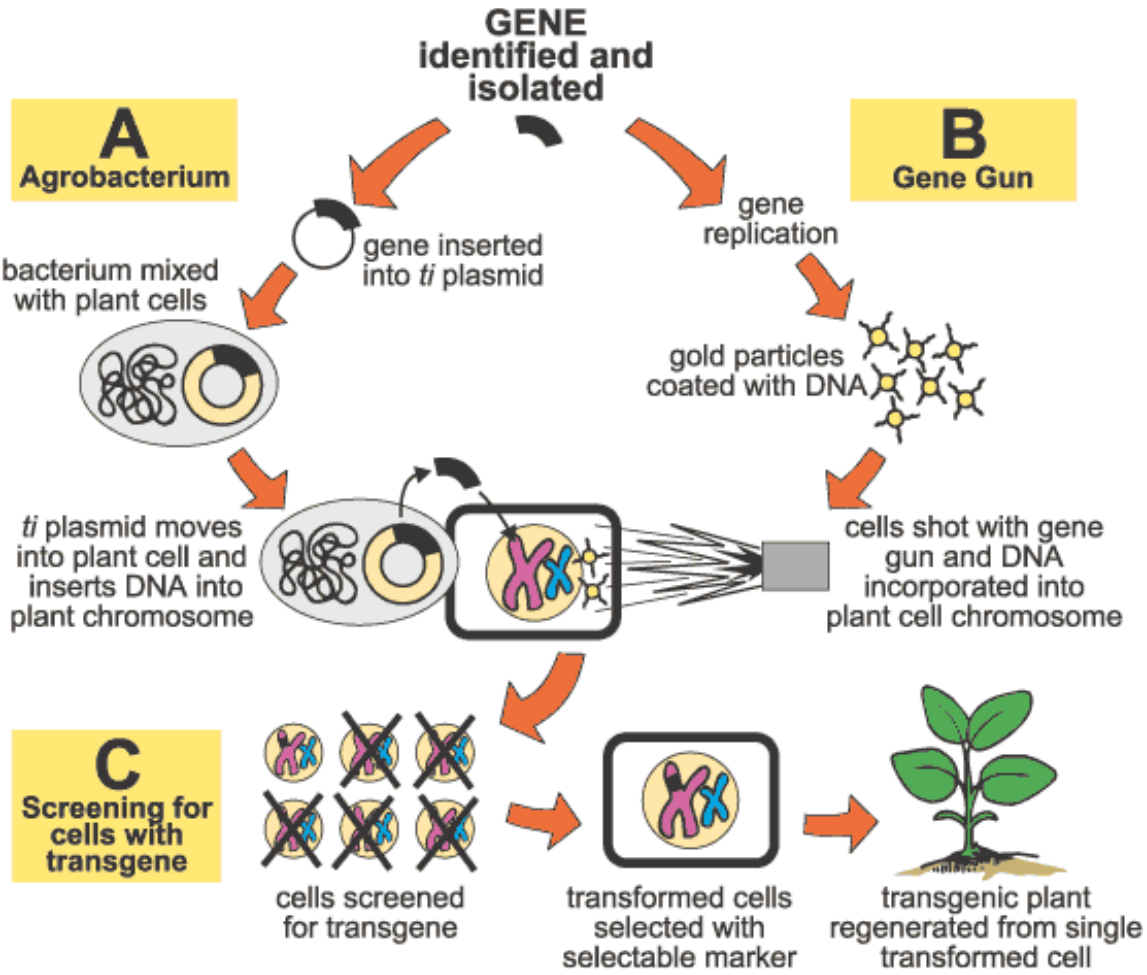
For development of transgenic of any crop, there are five important steps:

a. Identification of effective gene or genes
b. Gene transfer technology
c. Regeneration ability from protoplasts, callus or tissues
d. Gene expression of the product at desired level
e. Proper integration of genes so that are carried for generations by usual means of reproduction.

Once identification of bollworm inhibiting genes has been achieved, molecular biologists have step by step solved the problems to achieve perfect transgenics. In case of cotton, Agrobacterium-mediated gene transfer technique has been essentially used (Firozabady et al. 1987). Although now for direct gene transfer to protoplast, biolistic gene transfer techniques are available. The regeneration of cotton plants from callus and somatic embryogenesis has so far been restricted to few 'Coker' genotypes. All cotton genotypes are not amenable to regeneration and that is one big hurdle in gene transfer. There are reports of induction of somantic embryogenesis has also been reported from china and Australia but in India, attempts to repeat it with Indian genotypes have been unsuccessful. To circumvent the problem of genotype-limited regeneration of callus or leaf tissues, transformation and regeneration from meristematic tissues was attempted which was found useful. Using Cry 1 Ab and Cry 1 Ac genes, transgenic cottons with perfect integration,

expression and reproduction was achieved first in USA in 1987. Subsequently, there are reports from china and Australia.

There are four important methods of foreign gene (DNA) transfer in crop plants viz. plasmid method, particle bombardment, direct DNA uptake and micro-injection (Stewart, 1991). These methods are also known as systems of DNA delivery for genetic transformation. The soil borne bacterium *Agrobacterium tumifaciens* (termed as Nature's Genetic Engineering) is used for development of transgenic plants. This method has three main limitations viz. host specificity, somaclonal variation and slow generation. There are two main advantages of Agrobacterium mediated DNA transfer method. Firstly, this method has some control over the copy number and site of integration of transgene which is not possible in particle bombardment method. Secondly, this is a cheaper method of genetic transformation than particle bombardment method. Perlak et.al. (1991) transferred successfully the Cry 1 Ac gene to cotton via Agrobacterium with CaMV promoter and the Cry protein produces by transgenic cotton was found highly toxic to bollworms. This method was later used extensively by others.



The particle bombardment method in which the foreign DNA is delivered into plant cells through high velocity metal particles, has some advantages over the Agrobacterium mediated method of DNA transfer, This method does not exhibit host specificity. Hence, it can be effectively used for the development of transgenic plants in various plant species. Moreover, this method is

technically simple than Agrobacterium mediated DNA transfer method. In this method, there is no need of isolating protoplast.

The other two method viz. direct DNA transfer and microinjection technique are rarely used for developing transgenics in cotton. Currently, two DNA delivery system, viz.(1) Agrobacterium mediated gene transfer, and (2) bombardment of cells with plasmid DNA coated particles, are widely used for development of transgenic (genetically engineered) plants in cotton (Umbeck et al., 1987; Firoozbady et al., 1987; Finer and McMullen, 1990). More than 37 transgenic plants have been developed in cotton so far by these two methods.

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