What is 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 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 Technical Bulletin from CICR (www.cicr.org.in) 4 Transgenic Bt Cotton II proteins, which are specific to them. Other beneficial insects are unaffected by these proteins.

What is Bt Cotton?

A genotype or individual which is developed by the techniques of genetic engineering is referred to as transgenic.

In other words, genetically engineered organisms are called transgenics.

A transgenic may be a plant, an animal or a microbe. 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 <u>Bacillus thuringiensis</u>. The first transgenic plant was developed in 1983 in tobacco (Fraley et.al.1983) in U.S.A. In cotton, the first transgenic plant was developed in 1987 in U.S.A. by Monsanto, Delta and Pine companies (Benedict and Altman, 2001)

The transgenic cotton is of two types viz. (1) bollgaurd and (2) roundup ready cotton. The former confers resistance to bollworms and the latter is resistant to herbicides. The area under herbicide resistant transgenic cotton is resticted to USA. However, bollworm resistant Bt transgenic cotton has spread to several countries.

How Bt cotton is developed?

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.

In case of cotton, Agrobacterium-mediated gene transfer technique has been essentially used (Firozabady et al. 1987). Using Cry 1 Ab and Cry 1 Ac genes, transgenic cottons with perfect integration, expression and reproduction was achieved first in USA in 1987.

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). The first two workers used Agrobacterium method while the last workers used biolistic method of gene transfer in cotton for developing transgenic plants. More than 37 transgenic plants have been developed in cotton so far by these two methods.

What are the Benefits of Bt Cotton?

These benefits include direct benefits, such as reduced pesticide use, improved crop management effectiveness, reduced production costs, improved crop management effectiveness, reduced production costs, improved yield and profitability, reduction in farming risk and improvement opportunity to grow cotton in areas of severe pest infestation.

Indirect significant benefits of the technology include improved populations of beneficial insects and wildlife in cotton field, reduced pesticides runoff, air pollution and waste from the use insecticides, improved farm worker and neighbour safety, reduction in labour costs and time, reduction in fossil fuel use and improved soil quality.

The major advantage of Bt cotton are summarized below:

- The Bt cotton has inbuilt genetic resistance to bollworms and is very effective in controlling the yield losses caused by bollworms to a considerable extent. The resistance is governed by a single dominant gene.
- 1. Use of Bt cotton reduces use of pesticides resulting in reducing the cost of cultivation.
- 2. It results in improvement of yield levels and also improves margin of profit to the farmers.
- 3. It provides opportunities to grow cotton in areas of severe bollworm incidence.
- 4. It promotes ecofriendly cultivation of cotton and allows multiplication of beneficial insects i.e. parasites and predators of bollworms (Fitt et al. 1994, Luttrell and Nerzog, 1994).
- 5. It also reduces environmental pollution and risk of health hazards associated with use of insecticides because in Bt cotton the insecticides are rarely used. An average reduction of 3.6 sprays per crop season has been reported in Bt varieties as compared to non-Bt.

What are the Risks and Potential Impacts of Bt cotton on Human Health?

In the United States, the impacts of Bt cottons to human health have been investigated and approved prior to their use by the U.S.Food and Drug Administration (FDA).

Safety assessment of Bt cotton on human and animal health is science and risk-based and has focused on the following:

- A detailed understanding of the biology of cotton, including the uses of the products derived from cotton.
- A biochemical characterization of the introduced proteins, estimation of the levels of the protein in the important plant products, and a detailed assessment of the safety of the introduces proteins. The safety assessment includes: (1) a history of safe consumption of the proteins by humans or animals;(2) any prior animal toxicity testing of the proteins; (3) results from the field and lab safety studies to assess the allergic

effect, toxicity and digestibility of the expressed proteins, and (4) assessment of the dietary consumption of the proteins by humans and animals of cotton products.

- A determination of any unintended effects on the quality traits of the crop as a result of the insertion of the genetic material or the resulting protein expression. The concept is termed as 'Substantial Equivalence'. In cotton, testing of this concept included multiple location trials of agronomic characteristics and plant morphology, fibre quality, and nutritional components of the cottonseed oil and meal. These nutritional composition studies include proximates (protein, fat, carbohydrates, ash moisture and calories), fatty acid, spectrum, amino acid spectrum, and gossypol. Additionally, the equivalence of cottonseed oil and meal was also determined.
- Feeding studies with cottonseed or cottonseed meal were conducted with rats or other animals to determine any adverse health or behavioral effects.
- Review and testing of cotton products used in medical and personal hygiene products and food.

Now it is almost universally accepted that insects will eventually develop resistance to the toxin, thus, measures have already been adopted to delay the development of resistance.

A review of all safety information indicates that Bt cotton does not pose any different risk to human or animal health than conventional cotton. Each of the proteins introduced into Bt cotton commercialized to date has been shown not to require a tolerance level by the U.S. Environmental Protection Agency (EPA). This means these proteins are considered sage for human or animal consumption. Tolerance set by the EPA establish allowable, safe limits of pesticides in food (i.e. cottonseed oil) and feed (i.e. cottonseed, cottonseed meal, cottonseed hulls).

What are the Impacts on the Environment?

In the U.S., the U.S. Department of Agriculture (USDA) is responsible for field testing of all agricultural biotechnology crops. USDA evaluates whether a technology could pose a threat to plant or animal health. The U.S. Environmental Protection Agency (EPA) has regulatory authority for crops such as Bt cotton, which claim pesticidal properties (i.e. pest-protected plants).

Effect of Bt cotton on the health of animals, poultry, human and environment are summarized

below:

1. The feeding of Bt cotton seed to animal has not been reported to have any adverse effect.

2. Seed of Bt cotton and its cake do not have any adverse effect on digestion of animals. Moreover, no allergic or toxic effect of use of Bt cotton seed and meal has been reported.

3. The oil extracted from the seed of Bt cotton has not been found to have any adverse effect on human health.

4. No adverse effect of Bt cotton has been reported on non target beneficial insects so far.

5. The possibilities of cross pollination of Bt cotton to other species of Gossypium are nil to negligible because the Bt gene has been inserted in upland cotton (2n=52) which cannot outcross with cultivated or wild diploid cotton species (2n-26).

6. It can also not outcross with tetraploid wild species such as G.tomentosum which are found either in cultivated areas or extremely isolated species gardens maintained at different research institutes.

7. The upland cotton in which Bt gene has been inserted does not have cross compatibility with outer genera of the family of Malvaceae.

8. No adverse effect of Bt cotton on the environment has been reported by any of the countries where Bt cotton is commercially cultivated.

Where are we on Bt Cotton in India?

Basic Research:

In India, the basic research on Bt transgenic cotton is being carried out at the following research institutes / centres:

- 1. National Botanical Research Institute (NBRI), Lucknow
- 2. National Research Centre on Plant Biotechnology (NRCPB), New Delhi.
- 3. International Centre for Genetic Engineering & Biotechnology (ICGEB, New Delhi).
- 4. Central Institute for Cotton Research, Nagpur.
- 5. National Chemical Laboratory (NCL), Pune
- 6. Bhabha Atomic Research Centre (BARC), Mumbai, and
- 7. University of Agricultural Sciences, Dharwad.