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TRANSGENIC Bt COTTON

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1. Introduction

Cotton is one of the major fibre crops of global significance. It is cultivated in tropical and sub-tropical regions of more than eighty countries of world occupying nearly 33 m ha with an annual production of 19 to 20 million tones of bales. China, U.S.A., India, Pakistan, Uzbekistan, Australia, Brazil, Greece, Argentina and Egypt are major cotton producing countries. These countries contribute nearly 85% of the global cotton production. In India, cotton is being cultivated in 9.0 m ha and stands first in acreage. The crop is grown in varied agro-climatic situation across nine major states viz. Maharashtra, Gujarat, Madhya Pradesh, Punjab, Haryana, Rajasthan, Andhra Pradesh, Karnataka and Tamil Nadu. The crop is also grown on small area in Orissia, Assam, U.P and West Bengal. Nearly 60 million people are engaged in cotton production, marketing and processing. The textile industry which utilizes the cotton provides employment to about 16% of the total workforce. Cotton in its various forms also serves as raw material for more than 25 industries.

The decision of the Genetic Engineering Approval committee (GEAC) of Government of India clearing the release of Bt cotton for commercial cultivation during 2002-2003 crop season, is considered as one of the major milestones in the history of cotton improvement in India. Incidentally, cotton happens to be the first crop to receive environment clearance as GMO in Indian Agriculture, and thus has received maximum attention from planners, scientists, social workers, media, farmers and general public. With liberalization of world trade following WTO formation, quality and price competitiveness have become the buzz words not only for export performance but also for domestic use. India made significant strides in productivity since independence. The country was producing only 2.3 m bales of short and medium staple cotton from 4.4 million ha (with production of 88 kg lint / ha). With two major technological interventions viz. introduction of hybrid technology in early seventies and molecules in early eighties, productivity rose to 300 kg lint/ha. However, the protection technology has been misused and started showing negative impact, thus stagnating yields for the last 5-6 years. Today, productivity of Indian cottons is lowest in the world. In contrast, the major cotton producing countries have productivity 3 to 5 times higher. There are many reasons of low productivity of cotton in India. Besides dependency on 70% cotton production on vagaries on monsoon, diverse ecological and soil conditions, constant threat from pests and diseases is considered a major biological challenge to successful cotton productivity. Amongst the biotic stress factor, bollworms are by far the most serious pests of cotton and alternative controlling strategies, such as Bt cotton is considered a welcome technological step.

2. Why Bt Cotton?

In India, 162 species of insect pests attack different stages of cotton. Of these, about a dozen are major and half of them are key production constraints necessitating management interventions in the crop ecosystem. The sucking pest complex comprising of aphids, jassids, thrips and whitefly are widespread and fairly serious. However, their damage can be efficiently contained by the existing practices of cultural, chemical, biological and host resistance means. The bollworms are most important tissue feeders and highly damaging. Three types of bollworms viz. American bollworm (Helicoverpa armigera), Pink bollworm (Pectionphora gossypiella) and Spotted bollworm (Earias vitella), normally referred as bollworm complex are by far the most damaging and loss inducing pests of cotton. Amongst them, Helicoverpa emerged as a key pest all over the country causing as high as 80% losses in cotton.

The epidemic witnessed in Rajasthan, Haryana, Punjab during 2001-2002 season is estimated to have reduced the yield of cotton in North Zone of India by 50%. The frequent and regular crop failures in the last 15 years have been attributed to bollworms particularly Helicoverpa armigera. In India, an estimated Rs.33.8 billion to value of pesticides are used in agriculture (Agrolook, 2001), with Rs.16 billion worth on cotton alone. Bollworm control alone takes a heavy amount consting nearly Rs.12 billion and accounting for one third of current pesticide sales (Gupta et.al. 2001). Although, there has been some 10% reduction in pesticide consumption in cotton over the last 7 years, the crop with its only 5% area consumers the highest amount of pesticides (Table-1)

Table-1: Highly skewed pattern of pesticide use

Crop	Area(%) in India	Pesticide used in India (%)		Current pesticides used in world (%)
		1995	2001	
Rice	24	17.0	22.8	13.0
Cotton	05	54.0	44.5	10.2
Fruits + Vegetables	13	8.1	13.0	24.2
Oilseeds	10	2.2	03.5	N.A.

Although a wide variety of products are used to minimize the pest damage including bollworms, there are currently new serious problems such as resistance to pesticides, resurgence of secondary pests, environmental contamination due to indiscriminate use and unspecific, spurious product dumping in the market. To combat these problems, integrated pest management (IPM) with greater emhasis on biological control has been recommended in the absence of sound host resistance to bollworm. However, IPM requires training, demonstrations, making available biocontrol agents, participatory monitoring of pest incidence and community system approach at village level. Lot of efforts have been made for alternative strategies of bollworm management and various non-pesticidal (NPM), restricted pesticidal (IRM) and IPM techniques have been formulated, tested and demonstrated. These are slowly gaining acceptance but so far successes have been restricted and dissemination is slow. Transgenic Bt cotton, which evokes inbuilt resistance in the host is gaining wider adaptability as a means of avoidance of losses due to bollworm because the technology operates at seed level dissemination and find quick favour among the users. Therefore, Bt cotton has already proved useful in countries where it has been introduced earlier. In Indian context also, it is expected to give a wider base to all other protection strategies in cotton.

3. 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 protein 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. Other beneficial insects are unaffected by these proteins. The gene bank data base of Bacillus Genetic Stock Centre (BCSC) have given a list of Cry(Crystal), Cyt(Cytolytic) and Vip genes either synthetic or modified versions from B.thuringiensis. about 22 classes of Cry including 126 Cry genes have been registered along with a Crt gene and 3 Vip (Vegetative insecticidal protein) genes. But popularly and effectively utilized are Cry 1 Ac, Cry 1 Ab in different crops.

4. 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 Bacillus thuringiensis. 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).Later on, the research work on development of transgenic was intensified all over the globe and several transgenic plants were developed. 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. Transgenic disease resistant cottons have not yet been developed. Characterization of antifugal factors is underway at the USDA (Rajasekharan et.al.1999). In India, a few resistant genes against Fusarium and Verticillium wilts have been isolated and are being transformed into cotton. Chinese scientists have isolated ‘GO’ gene and have transformed them into cotton which have shown resistance to both the wilts (Zhang et.al.2000).

5. 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.

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 somantic embryogenesis have 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. In coming years, the techniques are being invented and the problems of genotype-dependent regeneration will be sorted out.

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.

6. How is Bt cotton different from conventionally bred cotton?

The scientific basis of plant breeding was established soon after the discovery of Mendel's laws of genetics. The basic concept of plant genetics is that the traits / characters are controlled by genes that genes are located on discrete structure called chromosomes located in the nucleus of each cell of an organism, and that mixing or recombination of parental genes occurs during the formation of sex cells in the first generation progeny. The number of different chromosomes in a cell is specific to the species. For example, in Upland cotton i.e. G.hirsutum and Egyptian cotton (G.barbadense) which are tetraplodis have 52 chromosomes while in the Indian diploid desi cottons, G.arboreum, G.herbaceum, the chromosome number is 26. In a normal plant, two copies of each chromosome are present. One set of chromosomes (say 26 in G.hirsutum) is contributed by a male parent and one set by the female parent. Two copies of each chromosome means that each gene is present in atleast two copies, although many genes may be present in multiple copies in the genome. A plant trait controlled by one gene pair such as fibre colour is called qualitative trait. However, most of the characters of agronomic or economic importance are quatitative and controlled by the interaction of many genes. For most traits different versions of the controlling genes exist. It is this diversity of gene type that provides the basis of traditional plant breeding. The breeder attempts to introduce a large number of genes (desirable) from a range of different genetic sources into a single superior genotype. Traditionally, it is done by sexual hybridization. Thus, gene transfer is limited to plants that are sexually compatible. While selecting for traits, a breeder has to eliminate the unwanted genes contributed by donor parent and thus involves the process of backcross, intercross, self pollination strategies and selections. Traditional breeding deals with large blocks of DNA and oftern a long drawn process to achieve

breakage of undesirable linkages, intense scrutiny to identify 'recombinant plants'containing only useful genes. These methods have been useful in enhancing the yield potential by more than 200 kg lint per ha in India on an average. Traditional breeding has also limited use in evolving bollworm-resistant cultivars as no precise sources of donors within compatible types are available for large scale breeding.

Genetic Engineering (GE) is a breeding strategy that attempts to avoid the problems associated with the transfer of large blocks of genetic material between two parents. The current state of technologies allows only a very limited number of foreign genes (from any life source) at a time to be introduced into a plant. However, single gene traits cause least disruption of the existing plant genome and are much easier to develop in subsequent breeding efforts. Two components are required to accomplish genetic engineering. The first is the knowledge of plant genomic structure and the structure of a single gene, and the second is the ability to develop a complete plant from a single cell (regeneration). Not all varieties can be regenerated so direct GE is limited to a few that can be. These are unfortunately not agronomically superior and hence a series of backcrossing and selection is required to put the new gene into the best varieties.

Genes are composed of DNA, a linear series of four basic chemical subunits. The linear order of these subunits determines the regulation and expression of genes. Each chromosome consists of one exceptionally long double stranded DNA molecule, and the genes are arranged linearly along the strand, usually with long stretches of non-functional DNA sequence, each with a different function, (1) a sequence at the start of gene called promoter, dictates when, where and how much of the gene product will be produced, (2) a central region, called the coding region, provides the genetic code for the gene product, and (3) a terminal region, where the gene ends. The final product of the gene, with few exeception, is generally a protein. The function of each gene / protein is specific, but collectively these functions range from nutrition storage and cell structure to metabolic catalysts (enzymes) and plant defensive agents. Protein with latter functions are used in Bt cotton lines.

In reality, dividual plant cell are 'transformed' by insertion of foreign DNA. Various techniques are available to do this, but the most common method relies on a system provided by nature. The bacterium causing the crown gall disease Agrobacterium tumefaciens is nature's own genetic engineer. It transfers some of its own DNA to plant cell as a part of the disease process. Scientists have removed the 'disease causing DNA' part from selected bacterial strains and discovered that the bacteria, while no longer causing the crown gall disease, retain the ability to transfer to a plant cell any DNA' that was removed. This natural system thus allows any gene to be transferred to a plant cell through this bacteria. Since the specific site on chromosome where new DNA is inserted into an existing gene, it is necessary to do many transformations and regeneration events and then select the transgenic plant that gives the best performance. This is called Agrobacterium-mediated gene transfer.

There are other methods of gene transfer available now, such as direct gene transfer to protoplasts and biolistic gene transfer where bombardment of regenerable tissues with DNA-coated microprojectiles at a very high velocity is used for ingestion of foreign genes into plant cells.

Traditional breeding methods deals with blocks of chromosomes based on sexual hybridization and recombination. GE deals with a very limited number of defined genes designed to impart traits to a crop that are not present in the traditional germplasm breeding pool.

7. What are the Benefits of Bt Cotton?

The introduction of Bt cotton has provided growers with a new tool for managing bollworms in cotton. Numerous benefits of this technology acrrue to the grower, the global cotton industry, and society on many levels-economic, environmental and social. 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 most signigicant benefit of biotech cotton to date has been the reduction in insecticidal usage for the control of certain bollworms. Numerous studies, conducted across the United States and in Australia, China, Mexico and Spain, have demonstrated an overall reduction in sprays for Lepidoptera pests. The number of spray reduction ranges from 1.0 to 7.7 sprays per crop season. An average reduction of 3.6 sprays per crop has also been proved by large scale DBT testing of MAHYCO of hybrids in India in 2000-2001 season.

Seven academies of science from around the world (the Royal Society of London, the U.S. National Academy of Sciences, the Brazilian Academy of Sciences, the Chinese Academy of Sciences, the Indian National Science Academy, the Mexican Academy of Sciences and the Third World Academy of Sciences) issued a report, Transgenic Plants and World Agriculture, in July 2000 spelling out the promise of agriculture biotechnology to alleviate hunger and poverty in the world. The paper urges governments to base their decisions regarding biotechnology on sound science and indicates that it will be critical to use the best science to make wise choices with respect to these technologies. It was pointed out that public health regulatory systems need to be put in place in every country to identify and monitor any potential adverse human health effects of transgenic plants, as is the case for any new plant variety. Likewise, environmental concerns must be addressed against the agricultural technologies currently in use that cause environmental problems. Procedures that most nations already have in place to approve the use of new crop plants could serve as the model for a more formal risk-assessment process.

Also the report, Genetically Modified Pest-Protected Plants: Science and Regulation, published in April 2000 by the U.S. National Academy of Sciences, found to be valid to the principles that “There is no evidence that unique hazards exist either in the use of recombinant DNA techniques or in the movement of genes between unrelated organisms” and “Assessment of the risks of introducing recombinant DNA engineered organisms into the environment should be based on the nature of the organism and the environment into which it is introduced, not on the method by which it was produced”.

The initial commercial GE cotton crops are designed primarily to deal with pests. To the extent that they reduce overall pesticide use, they reduce the potential for collateral damage to non-target species, including humans. Even if the effect of the technology is merely to substitute one pesticide for another, the net effect might be to reduce negative environmental consequences.

The major advantage of Bt cotton are summarized below:

1. 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 (Rummel et al. 1994, Flint et al. 1995, Bacheler and Mott, 1996). The resistance is governed by a single dominant gene.

2. Use of Bt cotton reuces use of pesticides resulting in reducing the cost of cultivation.

3. It results in improvement of yield levels and also improves margin of profit to the farmers.

4. It provides opportunities to grow cotton in areas of severe bollworm incidence.

5. 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).

6. 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.

8. 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). FDA is making this review mandatory prior to use and is establishing guidelines for voluntary labeling. Australia, Office of the Gene Technology Regulator, which coordinates assessments from the relevant health and environment authorities, also has robust regulatory requirements. Other countries and international groups do similar reviews prior to approval.

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.

Changes at the molecular level can be made to produce a particular compound that could trigger actions extremely important for the cotton industry. For instance, researches in the private and public sectors after many years of research developed a system which would case the plant to produce only infertile seed. This technique was named the Technology Protection System (TPS) and it means that farmers had to buy seed every year. TPS could be used not only with GE cotton but in other varieties too. The plans to commercialize TPS have been withdrawn, in part, because of public objections of the technology. GE could be used to produce genetic characteristics that might be objectionable by some farmers because of their traditional approaches to seed use and /or production practices.

Even before GE cotton became available, fears were expresses that insects could develop resistance to the toxin produced by the Bt gene. 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. The potential for resistance to develop in the target insects also means there is a need to routinely reengineer cotton with new genes that will produce toxins with different modes of action.

Currently, only two types of GE cottons involving three different genes have been commercialized and neither demonstrates any interaction with other genetic material in the cotton plant to produce deleterious effects. But, such interactions are not impossible.

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). Additional approvals for the use in food and feed of products derived from Bt cotton have been obtained following scientific review in Japan, Australia, Argentina, South Africa, Mexico, Canada and China. Scoured and bleached cotton, as it is used for medical and personal hygienic products as well as for chemical products, does not contain DNA or protein from a transgenic plant.

9. 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). EPA regulates (40 Code of Federal Regulations part 152.20) environmental exposure to these crops to ensure there are no adverse effects to the environment, non-target insects, and other organisms (e.g. microbes, earthworms and nematodes).EPA has announced that they will amend these regulations on the oversight of biological control agents by the end of 2000 to clarify how they regulate genetically engineered plant pesticides. Other countries and international groups conduct similar reviews prior to approving the use of Bt cottons.

The impact of Bt cotton on the environment has had science and risk-based assessments that have focused on the following components:

- Agronomic performance of all new cotton varieties is typically assessed through field observations to determine morphology, yield, lint quality, plant growth characteristics, and susceptibility to diseases and insects. These factors were all unaffected by the insertion of genetic material, except for the targeted differences in the proteins produced and the commensurate yield increases as an insect consequences.

- An assessment of the biology of Bt plants for pest or weediness potential relative to conventional cotton includes the potential for cross-pollination to weedy relatives, dormancy and germination changes, and overwintering potential. The inserted genetic material in these cotton products behaves as any other DNA that is transferred to progeny through Mendelian inheritance. For gene flow to occur via normal sexual transmission, certain conditions must exist; the two parents must be sexually compatible, their periods of flowering must coincide, a suitable pollen vector must be present and capable of transferring pollen between the two parents and resulting progeny must be fertile and ecologically fit for the environment in which they find themselves. Wild propulations of G.hirsutum are relatively rare and tend to be widely dispersed. Most grow in non-agricultural areas. Cotton is normally considered a self-pollinating crop, but can be cross-pollinated by certain insects. However, the possibility of cross pollination of the introduced genes from Bt cotton to other Gossypium species or to other plants of the same family is extremely low to nil for the following reasons and has been confirmed in cross-pollination studies.

  (i) Upland and Egyptian / Pima cotton has 52 chromosomes and is incompatible with cultivated or wild diploid cotton species having 26 chromosomes, and, therefore, cannot cross and produce fertile offspring.

  (ii) Although cross pollination to species having 52 chromosomes can occur, commercial cotton production generally does not occur in the same geographical locations where wild relatives are found. For example, cross pollination to G.tomentosum in Hawaii is possible, but no commercial cotton is grown in Hawaii.

  (iii) There are no identified species outside the cotton family that are sexually compatible with cultivated cotton.

- As assessment of impacts on non-target insect species has been conducted. Testing was conducted with the Bt protein due to its insecticidal properties. A large amount of testing has been conducted on the sprayable Bt products with demonstrated safety to non-target organisms. These results were confirmed for Bt cotton. The insects tested represent major insect classes and included adult and larval stages of honeybees, green lacewing, ladybird beetles, and parasitic Hymenoptera, as well as common soil organisms, earthworms and springtails. The absence of toxic effects in the non-target organism studies, even at the protein (Cry 1 Ac) levels considerably above the maximum predicted environmental exposure, demonstrate that Bt protein would not have adverse impacts on these and related non-target organisms. Additional field observation studies of impacts of Bt cotton on non-target organisms have shown increases in populations due to the reduction in non-specific pesticide use. Research with Bt the reduction in non-specific pesticide use. Research with Bt cotton on the persistence of these toxins, and their possible ecological and environmental effects in soil, demonstrated that the protein (Cry 1 Ab) is released in root exudates from Bt

corn grown in the lab and in natural soil in the field. However, no significant difference was observed in the amount of toxin in the soil between Bt corn and non-Bt corn, nor was there any effect on soil microbes, earthworms and nematodes, which are non-target species. The field study on the effect of Bt pollen on monarch butterflies showed that the concentration of Bt pollen adhering to milkweeds (the staple food of the larvae) within a few (1-5) meters of corn fields was typically too low to cause mortality of even small monarch caterpillars that might be present during pollen shed. Due to its large particle size (90-100 microns), most corn pollen deposits stay within the cornfield .Cotton pollen is in the same size range as corn pollen, however, cotton pollen is spiny and never transported by wind as corn is. Cotton pollen leaves a flower only when harvested for food by bees, and these are unaffected by Bt proteins. Non-target Lepidopteran larvae are not exposed to Bt proteins. Non-target Lepidopteran larvae are not exposed to Bt toxins from Bt cotton away from the plant itself. Any larvae that forages on the cotton is, by definition, a target pest. Adult butterflies and moths may visit a cotton field for nectar, but they do not eat pollen and nectar contains no protein.

- Am assessment of the environmental fate of the introduced proteins has been conducted. Soil degradation of the protein (Cry 1 Ac) alone or in cotton tissue was studied under both lab and field conditions, each showing rapid elimination of insecticidal activity in the soil, which was comparable to half-lives reported for microbial products.

Based on the low levels of environmental exposure to the introduced proteins and the data generated in the environmental safety assessments listed above, there are no anticipated adverse effects on the environment nor have any been reported since the introduction of Bt cotton in 1996 in any country where it is cultivated. Indeed, the most significant impact on the environment from the use of Bt cotton involves many of the benefits of the technology, such as reduced pesticide use.

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.

10. Where are we on Bt Cotton in India?

10.1 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.

The work on Bt cotton in India was first initiated in 1994 at CICR, Nagpur with World Bank aided Biotechnology Project. The Programme was undertaken with an objective to standardize the regeneration protocol in Indian cultivars. Large number of cultivated varieties from different agro-climatic zones were investigated for regeneration. Hypocotyl tissues from in-vitro germinated seedlings of cvs. LRA 5166, LRK 516, Bikaneri Narma, CNH 36, PKV 081, MCU 10, MCU 5, Suman, Khandwa 2, Khandwa 3, Coker 100 st, Coker UTT 68, Soneville 213 and some hybrids such as H4, H6, PKV Hy2 and NHH 44 were utilized for callus induction and regeneration. Callus induction was standardized in most of the cultivars and differentiation of roots from callus culture was also obtained. However, development of explants was hampered by browning. Strategy to reduce browing was developed by modifying carbohydrate source. Still complete regeneration of plantlets was not possible. Two cultivars, viz., PKV 081 and Khandwa 2 are identified to be embryogenic in nature but the frequency and recovery of plants from somantic embryo was very low. New approaches of callus induction and differentiation from anther / pollen were also successfully examined. There appeared certain factors for non-differentiation of callus into somantic embryos. Therefore, simultaneous regeneration of shoot tip and cotyledonary node were attempted. Several studies were carried out at CICR and a standard protocol best suited for Indian cultivars was developed for micropropagation and transformation using Agrobacterium mediated and particle gun gene transfer.

At CICR, tetraploid Indian elite cotton cultivars viz., LRK 516, LRA 5166, PKV 081, MCU 5, Khandwa 2 are being utilized for gene transfer by Agrobacterium-mediated gene transfer protocol LBA 4404, a strain of tumor-inducing bacteria carrying Cry 1 A (b) and Cry 1 A (c) genes have been used to transform shoot apical meristem and meristematic cells of cotyledonary node and embryonic axis. Both the explants are co-cultivated with Agrobacterium in MS medium fro 48 hours. Successful transformants were obtained especially in I RK 516 as tested positive using southern blot techniques. Diploid lines have also been regenerated and transferred with Cry 1 A (b). However, expression of Bt protein has been quite low and further repeated attempts are being made to improve the efficiency.

NBRI, Lucknow and the Centre for Molecular Biology, Delhi University. South Campus have synthesized Cry 1 A © indigenously by modifying certain codon and transferred the gene in Coker 312, a regenrable cultivar by Agrobacterium tumefaciens. The transgenic cotton plants are being grown as R 0 regeneration in glasshouse.

The CICR has developed its own technology of evaluation of Bt protein particularly the Cry 1 A © protein. The method of dipstick (quick detection) and ELISA using CICR developed antisera has been tested on large scale recently because of the illegal planting of Bt cotton (Navbaharat 151) in Gujarat. CICR is the only laboratory who has developed capabilities of such large scale, reliable testing. Even the Institute has trained personnels from Ministry of Environment and Agriculture Departments for field checking of Bt-cotton samples. It is thus, clear that when private seed companies are targeting only hybrid for transformation, the efforts of public institutions targeting varieties of both American and desi cottons will be rewarding in long run.

Efforts were already made to obtain successful transformations of Bt genes, Cry 1 A (b) and Cry 1 A (c) directly into local Indian cotton varieties such as LRA 5166 and LRK 516 as well as desi varieties at CICR, Nagpur. Several Indian cultivars which are amenable to regeneration through multiple shoot technique have been identified. There are serious efforts through DBT, NATP and ICAR sponsored projects to develop Indian Bt cottons. The transgenics developed are repeatedly evaluated for their effectiveness in more than one laboratory to be double sure of successful incorporation. Under NATP Project, currently three Institutes namely, NBRI, Lucknow; NRCPB New Delhi; and ICGEB New Delhi are engaged in synthesizing and processing of three new genes viz. Cry 1 Aa, Cry IF and Cry IA 5. Two organizations viz. CICR Nagpur and UAS Dharwad are trying to incorporate these indigenously developed genes individually as well as in combination. This transgenic will be of novel type. The insect is not expected to develop resistance because of combination of two genes. Some success in development of such transgenic cotton has already been achieved at CICR, Nagpur.

10.2 Applied Research:

A practical approach to commercialize Bt cotton in India was adopted by MAHYCO Company. Research in the area of Biotechnology involving the new technology of 'Gene splicing' (GMO's), is closely scrutinized and monitored by the Department of Biotechnology (DBT) and Ministry of environment and forest, Government of India. The DBT has set up several committee on Genetic Manipulation (RCGM) and Genetic Engineering approval Committee (GEAC) which involve experts from various fields and rather a interministeral set up finally to approve any GMO product in the country. RCGM also forms specific 'Monitoring and GMO product in the country. RCGM also forms specific 'Monitoring and Evalution committee (MEC)' for direct evaluation of field trials of crop products like Bt cotton. The Indian Regulatory Mechanism for Research and Development in GMO is summarized in flow chart by Khadi et.al (2002).

In case of Bt cotton, DBT approved MAHYCO (Maharashtra Hybrid seed Company), a premier Indian Seed Company in March 1995 to import 100 g. of the Bt cotton variety coker 312, transformed by Monsanto, U.S.A. and which contained Bt gene Cry 1 Ac. The Indian Company backcrossed this transgenic trait into elite parents of MECH hybrids. They followed the standard recurrent backcross breeding scheme to introgress a Bt gene, Cry 1 Ac expressing insecticidal crystal protein into their parents of hybrids (Benedict and Altma, 2001) for six generations and generated standard staple line extracting only Bt character from the source material. They were permitted the first limited field trial in 1997 in few cotton growing areas on plots of 200 m^2. In early 1998, the regulatory bodies permitted the company to conduct small scale trials. The trials were continued in 1999 to 2000 seasons and the company was asked to generate the requisite safety data. In June, 2001, the GEAC directed the company to conduct the trials for an other year and simultaneously directed them to conduct trials under the aegis of All India Coordinated Cotton Improvement Project. Accordingly, the Bt cotton trials were conducted under the AICCIP network

during rainy season of 2001-2002 to know the genetic potentiality of Bt and non-Bt MECH hybrids 12, 164 and 182, agronomic performance and reaction for insect pests and diseases in five trials each at eleven locations spread over six states of central and south zones. The trials have been highly encouraging (Tables 2-6). The field experiments revealed lower population of bollworm larvae in Bt hybrid than their counter non-Bt hybrid and conventional hybrid checks. Similarly, boll damage, locule damage, square damage were significantly less in Bt hybrids than other checks. The ETL for bollworm was crossed during 90-120 DAS on Bt hybrids while it crossed at 60-70 DAS on non-Bt and check hybrids. The reduction in number of sprays in Bt ranged from 2 to 4 sprays giving a cost reduction in spray amount ranging from Rs.3000 to Rs.4000. Besides increasing the cotton productivity, Bt cotton hybrids have also proved to be eco-friendly because reduction in pesticide sprays and marginal increase in natural enemy populations atleast at four locations. The GEAC approved the commercial release of these three Bt cotton hybrids of MAHYCO with certain specific terms and conditions and thus in the current crop season it has been allowed to be cultivated in farmers field under expert supervision.

Table-2: Increase in seed cotton yield in Bt hybrids over local checks

Hybrids	Central Zone	South zone
MECH 184 Bt	5.44	7.23
MECH 162 Bt	5.88	7.85
MECH 12 Bt	1.07	7.51

Table-3: Cost of plant protection and net profit in Bt over checks based on all trials.

Hybrids	% reduction	Net Profit (Rs. /ha.)
MECH 184 Bt	50.38	11,566
MECH 162 Bt	50.33	10,972
MECH 12 Bt	39.29	7,041

Table-4: Seed cotton yield of Bt cotton hybrids trial under AICCIP during 2001-2002

Entry	Seed cotton yield (q/ha)		
	Central zone	South zone	Mean
MECH 184 Bt	12.6	15.8	14.2
MECH 184 Bt	6.4	6.4	6.4
MECH 162 Bt	13.2	14.4	13.8
MECH 162 Bt	8.8	7.2	8.0
MECH 12 Bt	7.6	16.2	11.9
MECH 12 Bt	6.7	7.6	7.2
National check (NHH 44)	8.2	7.6	7.9

(iv) Based on 17 field trials at six locations in central zone and 13 field trials at 5 locations in south zone.

Table-5: Seed cotton yield of Bt cotton hybrids in AICCIP trials during 2001-2002 under total unprotected conditions*

Entry	Seed cotton yield (q/ha)		
	Central zone	South zone	Mean
MECH 184 Bt	9.4	10.2	9.8

MECH 184 Bt	4.9	5.6	5.3
MECH 162 Bt	10.2	8.9	9.6
MECH 162 Bt	6.2	4.3	5.3
MECH 12 Bt	4.2	7.6	5.9
MECH 12 Bt	3.7	4.6	4.2
National check (NHH 44)	6.2	3.9	5.1

*Based on 5 locations in central zone and 4 locations in south zone.

** The trials have been carried out without any seed treatment or spray for pest / disease control.

Table-6: Cost benefit analysis based on cost of plant protection in AICCIP trials in IPM trials*

Entry	Yield (q/ha)	Gross income (Rs/ha)	Cost of PP (Rs./ha)	% spent on PP	Net income (Rs/ha)	Extra income over check (Rs/ha)
MECH 184 Bt	14.00	25200	1413	5.61	23787	12630
MECH 162 Bt	13.67	24606	1413	5.74	23193	12036
MECH 12 Bt	11.67	21006	1727	8.22	19279	8122
National check (NHH 44)	7.31	13158	2001	15.21	11157	BASE

* Based on 9 location field trials in central and south zone.

11. How fat Bt cotton has spread?

The eara of transgenic cotton begain in 1990 with introduction of Cry 1A (b) and Cry 1A (c) genes into cotton plants and transformed plants showed high level of resistance to Helicoverpa. In USA, the first product 'bollgard' was launched in 1996 by the Monsanto company. The Bt cotton was tested extensively in the USA and approved by US Environmental Protection Agency (EPA), the USDA and US Food and Drug Administration. The field and lab tests proved that the transgenic cotton is highly effective against neonate larvae of Helicoverpa and Pectinophora. The toxin gene delivers the Bt protein directly to the neonates after they hatch and try to feed. The Bt gene from originally GE mother plant was transferred to advance cotton cultivars through backcrossing. The GE cotton in china has also been developed combining Bt and the cowpea trypsin inhibitor (CpT1) gene. Transgenic cottons resistant to lepidopteran pests are commercially cultivated in Australia, Mexico, china, USA and Argentina (Table-7). The maximum area under Bt cotton is in USA (72%) followed by south Africa (40%), Australia (30%) and Mexico (25%).

Table-7: Area of transgenic Bt cotton in different countries (2000-2001)

Countries	Total	Area under Bt cotton (m.ha.)	% area under Bt Cotton
U.S.A	4.40	3.93	72
China	4.00	1.00	20
Australia	0.45	0.15	30
Argentina	0.60	0.03	05
South Africa	0.10	0.04	40
Mexico	0.16	0.04	25

ICAC 1999 to 2001

In India, after extensive testing of Bt cotton in AICCIP and farmers field, government of India has approved commercial cultivation of Bt cotton with effect from 2002 crop season. The area

under Bt cotton, looking to its impact on bollworm control, is likely to increase in India in the years ahead. It has been given to understand that in the current year, Bt cotton is planted in 40,000 ha which is only 0.03% of the total area now. It is expected to grow to 2.5% in next year as number of other players in the field are coming forward (Mayee and Rao, 2002).

12. Future Thrusts

The genetic resistance is the cheapest and the most efficient method of protecting crop plants from pests. The Bt transgenic cotton with inbuilt genetic resistance to bollworms will help in protection of natural enemies of insect pests i.e. predators and parasites. It will also help in reduction the cost of cultivation by reducing the use of pesticides. Moreover, it will reduce environmental pollution and health hazards caused by pesticidal use. Transgenic cottons with Bt endotoxin protein does reduce expenditure on insecticides and create eco-friendly environment without reduction in yield. The future research work on Bt transgenic cottons needs to be directed towards following thrust areas:

2. Through widespread cultivation of Bt transgenic cotton, the main risk is development of insect resistance against Bt toxin. Hence, multiple sources of resistance should be identified and used in developing bollworm and herbicide resistant Bt transgenic cottons to avoid the risk of developing insect resistance and herbicide resistant weeds.

3. Recently, some transgenic Bt cotton hybrids have been released for commercial cultivation in India. The seed of these transgenic hybrids is very costly. The price of seed is Rs.1600/- for a packet of 450 g. which cannot be afforded by small and marginal farmers. Hence, there is need to provide Bt transgenic seeds at cheaper rate, which can be afforded by small and marginal farmers.

4. Cotton crop also suffers from abiotic stresses such as drought and salinity. There is need to develop Bt transgenic cottons with resistance to drought and salinity conditions.

5. In case of hybrids, the farmer has to purchase fresh seed every year at a very high cost. Hence, efforts should be made to develop Bt transgenic straight cotton varieties, the seed of which can be used by the farmers for 3-4 years.

6. Cotton is a fibre, oil and protein yielding crop. There is need to improve the quality of proteins and oil through genetic engineering besides fibre quality improvement.

7. Besides, Bt gene, several other genes can be used in future for developing resistant genotypes of cotton to various insects. For example, cholesterol oxidaze gene from Streptomycetes fungus can be used for developing boll weevil resistant genotypes.

8. The spider and scorpion venom genes can also be used for developing insect resistant genotypes of cotton.

9. The Helicoverpa armigera stunt virus contains three genes which attack midgut of Heliothis and ceases its feeding.

10. Protease inhibitor gene from cowpea, soybean and rhizomes of African Taro are being used for development of transgenic cotton.

11. Diploid cottons cover about 25% of cotton area in India. Hence there is need to develop transgenic Bt varieties and hybrids of diploid cotton.

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