

Background Note on Bt Cotton Cultivation in India

Under the Environment Protection Act (1986), the Ministry of Environment & Forests, has notified the Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells 1989 issued by the Ministry of Environment and Forests under the Environment (Protection) Act, 1986, These rules and regulations cover the areas of research as well as large scale applications of GMOs and products made there from throughout India. The rules also cover the application of hazardous microorganisms which may not be genetically modified. Hazardous microorganisms include those which are pathogenic to animals as well as plants. The rules cover activities involving manufacture, use, import, export, storage and research. The target substances covered are, besides the hazardous natural microorganisms, all genetically engineered organisms including microorganisms, plants and animals.

These rules also define the competent authorities and composition of such authorities for handling of various aspects of the rules. Presently there are six competent authorities that is , Recombinant DNA Advisory Committee (RDAC), Institutional Biosafety Committees (IBSC), Review Committee on Genetic Manipulation (RCGM), Genetic Engineering Approval Committee (GEAC), State Biotechnology Coordination Committee (SBCC) and the District Level Committee (DLC). The RCGM established under the Department of Biotechnology supervises research activities including small scale field trials, whereas approvals for large scale releases and commercialization of GMOs are given by the GEAC, established under the Ministry of Environment and Forests. The Rules also mandate that every institution engaged in GMO research establish an IBSC to oversee such research and to interface with the RCGM in regulating it.

The Government is following a policy of case by case approval of transgenic crops. Introduction of any new technology requires careful evaluation and long term sustainable benefits. Bt. Cotton is the first and only transgenic crop approved by GEAC for commercial cultivation in 6 States namely Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu.

Cotton is a leading commercial crop grown for its valuable fiber. India ranks number one in the world accounting for 20% of the total area planted under cotton. However, even with highest area under cotton, nine million hectares, India ranks only third position with only 13% in production of cotton. India's average yield is only 319 kg/ha lintas compared to world average of 603 kg/ha. Cotton is highly susceptible to insects; especially to the larvae of lepidopteran pests, which is impacting cotton production. Fifty percent of the total insecticides consumed in the country are used only for cotton crop. The total loss due to damage to cotton crop is estimated to be more than Rs.1200 crores. The chemical control to suppress these insect pest are proving

ineffective as these pests have developed high level of resistance for most of such chemical used for the control of bollworm complex. Such a high level of resistance require repeated application of insecticides leading to heavy expenditure, crop failures, and vicious cycle of debt for farmers. Therefore, it has been argued that adoption of Bt cotton could help in protecting the crop against potentially the most damaging bollworms and thus reduce the risk of crop failures.

Bt cotton, a transgenic plant, produces an insect controlling prote in Cry1A(c), the gene for which has been derived from the naturally occurring bacterium, *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k.). The cotton hybrids containing Bt gene produces its own toxin for bollworm attack thus significantly reducing chemical insecticide use and providing a major benefit to cotton growers and the environment.

Bt cotton contains the following three genes inserted via genetic engineering techniques:

- The *Cry1Ac* gene, which encodes for an insecticidal protein, Cry1Ac, derived from the common soil microbe *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k.).
- The *nptII* gene, which encodes the selectable marker enzyme neomycin phosphotransferase II (NPTII), was used to identify transformed cells that contained the Cry1Ac protein. It served no other purpose and has no pesticide properties. The *nptII* gene is derived from the prokaryotic transposon Tn5.
- The *aad* gene which encodes the bacterial selectable marker enzyme 3''(9)-O- aminoglycoside adenytransferase (AAD) allowed for the selection of bacteria containing the PV-GHBK04 plasmid on media containing spectinomycin or streptomycin. The *aad* gene was isolated from transposon Tn7.

NPTII and AAD proteins are used as a selectable marker and have no pesticidal activity and are not known to be toxic to any species.

Bt cotton being a transgenic crop requires environmental clearance under Rule 7-10 of the 1989 "Rules for Manufacture, Use, Import, Export and storage of hazardous microorganisms/Genetically Engineered Organisms or Cells" notified under the Environment (Protection) Act, 1986. Prior to the deregulation of Bt cotton, enough data and information is necessary to evaluate its performance and environmental safety. The bio-safety and environmental issues related to this novel from were assessed, which includes molecular characterization of induced gene, biochemical characterization of the expressed protein, estimation of the level of the expressed proteins in cotton, proteins in cotton products, safety of the expressed proteins to non-target organisms, environmental fate of the Bt protein, and agronomic, compositional and food and feed safety evaluation of Bt cotton compared to non-Bt cotton seed.

The GEAC set up under the 1989 Rules accorded conditional approval for introduction of three Bt cotton hybrids namely BT MECH 162, BT MECH 184, BT MECH 12 into the environment in March, 2002 after detailed evaluation on the efficacy and safety of the product.

Conditions Stipulated by GEAC:

- (i) The period of validity of approval is three years from April 2002 – March 2005.
- (ii) Every field where Bt cotton is planted shall be fully surrounded by a belt of land called 'refuge' in which the same non-Bt cotton variety shall be sown. The size of the refuge belt should be such as to take at least five rows of non-Bt cotton or shall be 20% of total sown area whichever is more.
- (iii) To facilitate this, each packet of seeds of the approved varieties should also contain a separate packet of the seeds of the same non-Bt cotton variety which is sufficient for planting in the refuge defined above.
- (iv) Each packet should be appropriately labeled indicating the contents and the description of the Bt hybrid including the name of the transgene, the GEAC approval reference, physical and genetic purity of the seeds. The packet should also contain detailed directions for use including sowing pattern, pest management, suitability of agro-climatic conditions etc., in vernacular language.
- (v) MAHYCO will enter into agreements with their dealers/agents, that will specify the requirements from dealers/agents to provide details about the sale of seeds, acreage cultivated, and state/regions where Bt cotton is sown.
- (vi) MAHYCO will prepare annual reports by 31st March each year on the use of Bt cotton hybrid varieties by dealers, acreage, locality (state and region) and submit the same in electronic form to GEAC, if asked for by the GEAC.
- (vii) MAHYCO will develop plans for Bt based Integrated Pest Management and include this information in the seed packet.
- (viii) MAHYCO will monitor annually the susceptibility of bollworms to Bt gene vis-à-vis baseline susceptibility data and submit data relating to resistance development, if any, to GEAC.
- (ix) Monitoring of susceptibility of bollworms to the Bt gene will also be undertaken by an agency identified by the Ministry of Environment and Forests at applicant's cost. The Ministry has entrusted Central

Institute for Cotton research, Nagpur to carry out the above monitoring.

- (x) MAHYCO will undertake an awareness and education programme, interalia through development and distribution of educational material on Bt cotton, for farmers, dealers and others.
- (xi) MAHYCO will also continue to undertake studies on possible impacts on non-target insects and crops, and report back to GEAC annually.
- (xii) The label on each packet of seeds, and the instruction manual inside the packet should contain all relevant information.
- (xiii) MAHYCO will deposit 100 g seed each of approved hybrids as well as their parental lines with the National Bureau of Plant Genetic Resources (NBPGR).
- (xiv) MAHYCO will develop and deposit with the NBPGR, the DNA fingerprints of the approved varieties.
- (xv) MAHYCO will also provide to the NBPGR, the testing procedures for identifying transgenic traits in the approved varieties by DNA and protein methods.

The chronology of Bt cotton development by MAHYCO is as follows:

1994	Formation of IBSC and application for transgenic Bt cottonseed import.
1995	Permit from DBT received to import 100 g. Bt Cotton seed of Coker 312 from Monsanto, USA.
1996	Imported seed and Green House trial initiated.
1996	Limited field trial (1 Location) to assess pollen escape.
1996	Back crossing (Ongoing) breeding for transfer of Bt gene into elite parental lines in green house.
1997/98	Limited field trials (5 locations) to assess pollen escape.
1998	Toxicological (Ruminant goat model) and Allergenicity (BNR model) studies.
1998/99	Multi-centric research trials (15 +25 locations) to assess efficacy of Bt gene in Indian elite germplasm.
1999	Multi-centric research trials (11 locations) to assess efficacy of Bt gene in Indian elite germplasm.
2000/01	(a) Large-scale trials (100ha) to assess efficacy of Bt gene in Indian elite germplasm and the performance of Bt hybrids. (b) Hybrid seed production (150 ha) (c) Various biosafety studies. (d) ICAR trials at 6 locations.

- 2001-02- (a) Large-scale trials (100ha) to assess efficacy Bt gene in Indian elite germplasm and the performance of Bt hybrids,
 (b) Hybrid seed production (300 ha)
 (c) Biosafety studies.
 (d) ICAR Trials at 11 locations.
- April 2002 **Commercial cultivation in Six States (Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu)**

Biosafety Assessment:

The environmental safety assessment of Bt cotton hybrids include: pollen escape out- crossing, aggressiveness and weediness, effect on non-target organisms, presence of Cry 1AC protein in soil, effect of Cry1 AC protein on soil micro-flora, confirmation of the absence of Terminator Gene, and baseline susceptibility studies. MAHYCO conducted these studies as per the Protocol approved by the Review Committee on Genetic Manipulation functioning in the Department of Biotechnology. Brief note on the biosafety assessment is summarized below:

Studies conducted on pollen escape/out crossing

Multi-location experiments conducted in 1996, 1997 and 2000 revealed that out-crossing occurred only upto 2 meters, and only 2% of the pollen reached a distance of 15 m. As the pollen is heavy and sticky, the range of pollen transfer is limited. Also there is essentially no chance that the Bt gene will transfer from cultivated tetraploid species such as the present Bt hybrids to traditionally cultivated diploid species.

Aggressiveness & Weediness

To assess the weediness of Bt cotton the rate of germination and vigor was compared by laboratory test and in soil to the non-transformed parental line. The results demonstrated that there are no substantial differences between Bt and non-Bt cotton for germination and vigor. This also indicates that there is no substantial difference between transgenic Bt and control non-Bt cotton with regard to their weediness potential.

Studies conducted on the effect of Bt on non-target organisms

Studies conducted during the multi-location field trials revealed that the Bt cotton hybrids do not have any toxic effects on the non-target species, namely sucking pests (aphids, jassids, white fly and mites). The population of secondary lepidopteran pests, namely tobacco caterpillar remained negligible during the study period in both Bt and non Bt hybrids. The beneficial insects (lady beetle, spiders) remained active in both Bt and non Bt varieties.

Studies conducted regarding presence of Bt gene in soil

Studies were conducted to assess the possible risk of accumulation of Bt gene in the soil, by insect bioassays. Bt protein was not detected in soil samples indicating that Bt protein is rapidly degraded in the soil on which Bt cotton is grown. This study showed that the Cry 1AC protein was rapidly degraded in the soil in both the purified form of the protein and as part of the cotton plant tissue. The half life for the purified protein was less than 20 days. The half-life of the Cry 1AC protein in plant tissue was calculated to 41 days which is comparable to the degradation rates reported for microbial formulations of Bt.

Studies to evaluate the effect of Bt gene on soil micro-flora

Studies were conducted to evaluate any impact of Bt protein leached by roots of Bt cotton on the soil micro-flora. There was no significant difference in population of microbes and soil invertebrates like earthworm and Gllembola between Bt and non-Bt soil samples.

Studies to evaluate the Food Safety

For evaluating food safety, the studies conducted include: compositional analysis, allergenicity studies, toxicological study, presence of Bt gene and protein in Bt cottonseed oil and feeding studies on fish, chicken, cows and buffaloes. Salient features of these studies are as follows:

➤ **Compositional analysis**

The studies revealed that there is no change in the composition of Bt and non Bt seeds, with respect to proteins, carbohydrates, oil, calories and ash content.

➤ **Allergenicity studies**

Allergenicity studies were conducted in Brown Norway rates. No significant differences in feed consumption, animal weight gain and general animal health were found between animals fed with Bt cotton seed and no cottonseed. At the end of the feeding period, the relative allergenicity of traditional cotton hybrids and Bt cotton were compared to Bt and non-Bt Protein extract in active cutaneous anaphylaxis assays. Results of the study concluded that there is no significant change in endogenous allergens of Bt cottonseed compared to non-Bt cottonseed.

➤ **Toxicological study**

A goat feeding study was conducted for understanding the toxicological effects of Bt cottonseed. The animals were assessed for gross pathology and histopathology. No significant differences were found between animals fed with Bt and non Bt cottonseed.

➤ **Presence of Cry 1AC gene and protein in Bt cottonseed oil**

Studies have indicated that Cry 1AC gene and protein are not found in refined oil obtained from Bt cottonseeds.

➤ **Feeding studies on fish, chicken, cows and buffaloes**

Feeding experiments conducted with Bt cotton seed meal on fish chicken, cows and buffaloes indicated that Bt cotton seed meal is nutritionally equivalent, wholesome and safe as the non-Bt cottonseed meal.

The feeding experiments on poultry, fish, cows and buffaloes were conducted at National Dairy Research Institute (NDRI), Karnal on lactating cows; Department of Animal Nutrition, College of Veterinary Sciences, G.B. Pant University of Agriculture & Technology, Pantnagar on lactating buffaloes; Central Avian Research Institute (CARI), Izzatnagar on poultry; and Central Institute of Fisheries Education (CIFE), Mumbai on fish.

Confirmation of the absence of “Terminator Gene”

The transgenic Bt Cotton plant was developed by incorporating *Bt* gene into it. Therefore it was desirable to assess that no other gene including *cre recombinase* gene which is an integral component of the so called “terminator technology” is present in Bt cotton. A study was carried out by The Department of Genetics, University of Delhi (South Campus) Delhi to check the presence/absence of such gene in the Bt cotton. The PCR analysis of DNA samples isolated from individual seedlings derived from Bt cotton hybrids showed that Bt cotton hybrid lines positive for *Cry1Ac* amplification did not show any amplification product using *cre* primers. This conclusively demonstrated the absence of “terminator gene” in Bt cotton hybrids.

Baseline susceptibility study

The Project Directorate of Biological Control, Bangalore carried out baseline susceptibility study for *Helicoverpa armigera*, for two years in 2000-01 and 2001-02 at six and fourteen locations, respectively. In the study different geographical populations of American bollworm (*Helicoverpa armigera*) collected from six major cotton growing states of India (viz. Madhya Pradesh, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamilnadu) were exposed to insecticidal protein Cry1Ac through bioassays using probit analysis. Lc50 ranged from 0.114 to 0.594, Lc90 from 1.016 to 6.700 and Lc95 from 2004 to 19.462. However, the molt inhibitor concentration (MIC) ranged for MIC50 from 0.051 to 0.140, MIC90 0.246 to 0.910 and MIC95 from 0.024 to 1.826 of Cry1Ac. These values are from the baseline data for susceptibility of American bollworm to Cry1Ac protein and can be used as benchmark for monitoring resistance in the bollworm pest to Cry1Ac protein, in future.

Performance of Bt Cotton during Kharif 2002.

1. For Kharif 2002, the Committees constituted by the Ministry visited some of the Bt Cotton fields in the states of Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh with a view to monitor the compliance stipulated by the GEAC with respect to planting of 5 rows of non-Bt refuge, quantity of pesticide sprayed, farmers view's etc. The committee's observations and discussions with the farmers indicated that the performance of Bt cotton as compared to its non Bt counterpart is satisfactory in terms of bollworm infestation and reduction in use of pesticides spray.
2. However, in view of the numerous adverse newspaper reports regarding failure of Bt cotton, the respective State Dept of Agriculture were requested to submit a detailed report on the performance Bt Cotton as well as information pertaining to the total area under cotton cultivation vis-à-vis area under Bt Cotton, total area under irrigation, yield from Bt Cotton field vis-à-vis non-Bt fields, use of pesticides, accrued agronomic benefits, etc. The performance report from the respective State Govt. after harvest of Bt cotton has been received. The performance of Bt cotton has also been evaluated by the CICR
3. Analysis of the various reports indicates that, the performance of Bt cotton cannot be established based on one year data. It has therefore been decided to evaluate its performance for a period of three years. For the Kharif 2003, the Ministry has constituted a Committee comprising of members from Ministry of Agriculture, ICAR, GEAC, Central Cotton Research Institute, DBT and MoEF with the following Terms of Reference to institute a proper monitoring and verification mechanism.
 - Formulate a mechanism for verification of seed production and distribution and ensure quality control.
 - Formulate a mechanism for monitoring the performance of Bt cotton in the six states. The Committee will decide on the parameters to be monitored.
 - Constitute monitoring teams to monitor the Bt Cotton fields in the six states. The Committee will decide the appropriate time for monitoring in each State.
 - Standard format for submitting the monitoring report.
 - Information to be provided/collected from the respective State Agriculture department.
 - Mechanism for monitoring illegal release.

The following documents may be viewed at <http://envfor.nic.in>

1. **Reports of the Monitoring Committee constituted by the Ministry.**
2. **Reports of the respective State Govt.**
3. **Report of the Central Institute for Cotton Research, Nagpur.**
4. **Monitoring Mechanism for Kharif, 2003.**
5. **Stakeholder perception (under preparation).**
6. **Verification Report of CICR on spurious Bt. Cotton seeds in Gujarat.**

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