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HANDBOOK FOR SEED INSPECTORS

Genetically Modified Seeds and Regulations

Prepared under

gef UNEP

Phase-II Capacity Building Project on Biosafety

Ministry of Environment
Forests and Climate Change

Ministry of Environment
Forests and Climate Change
Government of India

In association with

BCIL
Biotech Consortium India Limited
New Delhi

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Prepared by

Ministry of Environment, Forest and Climate Change (MoEFCC) and
Biotech Consortium India Limited, New Delhi
under the UNEP/GEF supported Phase II Capacity Building Project on Biosafety

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Introduction

Genetically modified (GM) seeds/plants are developed by application of modern biotechnology wherein basic genetic material (DNA) has been altered using genetic engineering (GE) techniques. These are also referred to as GM crops, GE plants or transgenic crops.

In most cases, the aim is to introduce a new trait to the plant such as pest resistance, disease resistance, herbicide tolerance, improved product quality etc. As of 2017, 16 GM crops have been approved for commercial cultivation in the world. The global area under cultivation of GM crops has increased to 185 million hectares in 2017 from 1.7 million hectares in 1996 when the first GM crop was commercially cultivated.

In India, Bt cotton containing the cry1Ac gene from *Bacillus thuringiensis* was approved by Government of India in March 2002. The total area under Bt cotton has increased from 0.05 million hectares in 2002 to 11.4 million hectares in 2017. Bt cotton is cultivated in more than 90% of the area under cotton cultivation in India. Bt brinjal and GM mustard have also undergone extensive safety assesssment process and are under regulatory consideration.

As with any new emerging technologies, safety concerns have been expressed for GM crops as these may contain genes that have crossed the species barriers as compared to classical selection techniques used in traditional breeding. These include potential risks associated with their impact to human health, environment and biological diversity. Therefore, countries with active biotech programmes have put in place biosafety regulatory framework for GM crops.

In India, GM crops are regulated as per Rules for the Manufacture, Use/Import/Export and Storage of Hazardous Microrganisms/ Genetically Engineered Organisms or Cells (Rules, 1989) notified in 1989 under Environment (Protection) Act, 1986. All the steps in the development of

GM crops viz. laboratory research, greenhouse/ nethouse studies, confined field trials and safety studies are regulated before granting permission for environmental release. The regulatory committees function under the Ministry of Environment, Forest and Climate Change (MoEFCC) and Department of Biotechnology (DBT) along with the state governments. Seed certification department, seed inspectors, seed testing laboratories and other concerned officials have an extremely important role to play in ensuring implementation of biosafety regulations for safe and sustainable use of GM crops. Accordingly they have been empowered under Rules, 1989 for effective monitoring.

MoEFCC has implemented Phase II Capacity Building Project on Biosafety with support from Global Environment Facility (GEF) through the United Nations Environment Program (UNEP), to strengthen the biosafety management in India. 'Enhancing Public Awareness' is one of the key thrust areas of the project and is essential for better understanding of the biosafety regulatory framework. Accordingly, several knowledge products have been developed as part of the project. Significant efforts have been made to ensure outreach through multiple tools viz. workshops, printed material, short film etc.

In continuing with the same, MoEFCC in association with Biotech Consortium India Ltd. (BCIL), the project coordination unit has prepared booklet for specific categories of stakeholders focusing on their information requirements.

This handbook has been prepared to inform seed inspectors and concerned stakeholders about the key aspects of GM crops. It has the following eight sections:

1. Development of GM crops
2. GM crops: Applications and status
3. Safety assessment of GM crops
4. Regulations of GM crops in India
5. Cartagena Protocol on Biosafety
6. Confined field trials of GM crops
7. Detection of GM crops
8. Role of state agencies in monitoring of GM crops

Section 1: Development of GM Crops

Selective breeding and cross-fertilization have been used for thousand of years to impart desirable traits in plants such as higher yields and resistance to pests. New technologies and guidance on management practices is required regularly not only to increase the productivity, but also to deal with abiotic and biotic stresses such as drought, salinity, diseases etc.

Recently genetic engineering techniques have been used for introduction of desired traits. Genetic engineering allows transfer of genes from a wide range of living sources for development of superior plant varieties. This is important breakthrough, as characteristics of interest do not always exist in related species and therefore cannot be transferred using conventional breeding techniques. Introduction of useful genes from a wide range of organisms is possible because the genetic code is universal i.e. the DNA[1] of all organisms is made up of the same building blocks and is encoded in exactly the same way. A copy of DNA sequence (or gene[2]) encoding a particular characteristics can be therefore transferred into the cell of a different organism.

Once the gene is incorporated into the genome of a plant recipient, the resulting plant is considered to be genetically modified (GM)/ genetically engineered (GE)and the new characteristics coded by that gene are inherited by subsequent generations. The development of a GM crop is a multistep process as depicted in Figure 1 and explained below:

- **Identification of genes:** The first step is the identification of a gene(s) responsible for a desired trait in an organism (plant, animal or microorganism) followed by isolation and copying the gene of interest by use of molecular biology techniques.
- **Designing genes for insertion:** Once isolated and cloned, the gene of interest has to be modified with additional components (referred as gene construct) before it can

[1]DNA: Deoxyribonucleic acid, more commonly known as DNA, is a complex molecule that contains all of the information responsible for the inheritance of traits such as size, shape, color, build and other physical attributes of microorganisms, plants, animals and humans. DNA exists in the nucleus of each cell.

[2]Gene: A gene is basically a discrete segment of DNA encoding for set of instructions in the cell and contains all information concerning the form and functions of all living cells that give characteristics to an organism.

be effectively inserted into host plant. These may include addition of a promoter and termination sequences to signal the initiation and completion of sequence of gene of interest and marker gene for identification of GE cells/tissues during experimental process.

- **Transformation:** The gene construct is then transferred to the host plant through process of transformation using a Gene Gun method or the Agrobacterium method. Transformation is a heritable change in a cell or organism brought about by the uptake and establishment of introduced gene in the host plant.
- **Selection:** Following the gene insertion process, plant tissues are transferred to a selective medium (such as containing an antibiotic or herbicide), depending on the type of selectable marker used. Only plants expressing the selectable marker gene will survive indicating that they possess the transgene of interest. The whole plants are generated using tissue culture methods for further evaluation in laboratories and green houses. The evaluation includes activity of the introduced genes, stable inheritance of the genes and any unintended effects on plant growth, yield quality etc.
- **Field Trials and Safety Assessment:** The next step in the process is multi-location and multi-year evaluation trials in greenhouse and field environment to test the effects of the transgene and its overall performance. This phase also includes evaluation of environmental effects and food safety.

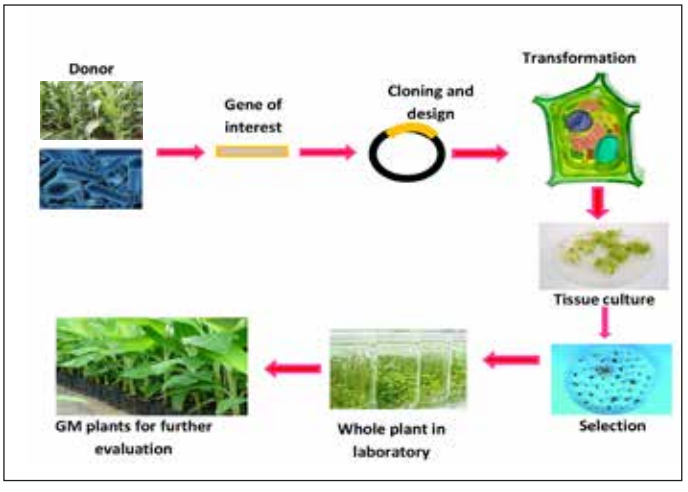


Fig 1: Development process of a GM crop

All data/ information generated experimental trials and studies are evaluated by regulatory authorities before granting permission for environmental release or commercialization.

Section 2: GM Crops: Applications and status

GM crops have been developed to incorporate various traits such as insect/pest resistance, herbicide tolerance, disease resistance, altered nutritional profile, enhanced storage life etc. The benefits may include:

- higher crop productivity due to reduced loss to pests and diseases
- reduction in farm costs and thereby increase in farm profit
- general improvement in health and environment due to availability of nutritionally enhanced food
- reduced use of pesticides/ insecticides in the environment which would further reduce the fuel consumption and also led to preservation of natural resources like soil and water due to decreased tillage
- improved weed control due to use of herbicide resistant GE plants

Potential benefits of various traits incorporated in the GM crops are summarized in Figure 2 below:

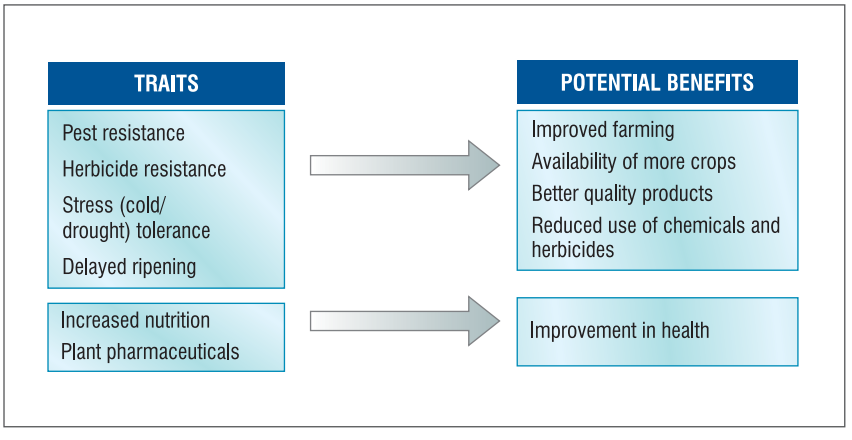


Fig 2: Potential benefits of GM crops

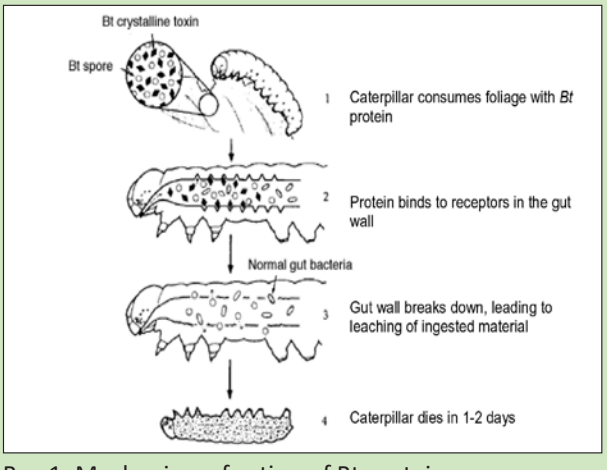
Plants that have been subjected for genetic improvement to multiple traits include several commercially important crops such as maize, soybean, tomato, cotton, potato, mustard and rice; horticultural plants such as papaya, plum; grasses such as alfalfa and trees such as poplar.

Most common traits so far include insect resistance and herbicide tolerance. Other traits of interest include virus resistance, fungal resistance, high nutritional value, improved product quality etc.

Some of the commercially cultivated GM crops in various countries are as follows:

i) **Bt Cotton** contains a built in insecticidal protein from naturally occurring soil microorganisms *Bacillus thuringiensis* (Bt) that gives protection to cotton from budworms and bollworms. Bt protein reduces or eliminates the need for additional insecticide applications for these pests. Bt is effective only to target pests but does not harm humans, animals, fish, birds and beneficial insects, by virtue of highly specific mechanism of action. The mechanism of Bt protein is given in the Box 1.

Bt cotton and Bt maize which have increased resistance to bollworms have been developed and cultivated since 1996.



Box 1: Mechanism of action of Bt protein

The spores of *Bacillus thuringiensis* (Bt) contain a crystalline protein (*cry*), which breaks down to release a protein, known as delta-endotoxin, which is highly toxic to lepidopteran larvae. Different *cry* genes, also known as Bt genes have been identified, cloned and characterized. Effective gene constructs have made it possible to deliver these genes into plant tissues so that they are expressed at levels high enough to kill the target insects.

ii) **Herbicide tolerant (HT) Soybean** contains a gene that provides resistance to selective herbicides. As many effective broad spectrum herbicides do not distinguish between weeds and crops, crops have been modified to make them resistant to herbicides so as to eliminate weeds more selectively. For example, HT soybean resistant to herbicide roundup has been developed. When the herbicide is sprayed, it kills the weeds but has no effect on soybean plants. Therefore, the herbicide can be applied in a single dose or fewer doses of concentration, thereby reducing farming cost and environmental damage. It provides better weed control, thereby improves farm efficiency by optimizing yield and saving time for farmers.

iii) **Virus resistant Papaya** contains a viral gene that encodes for the coat protein of papaya ringspot virus (PRSV). It provides the papaya plant with built in protection against PRSV.

iv) **GM Maize** has been developed incorporating both insect resistant and herbicide tolerant genes. Both the traits have also been incorporated in varieties through stacking. Drought tolerant maize containing genes to maintain normal physiological performance during stress events has also been approved for commercial cultivation.

v) **Hybrid Canola** contains transgenes for a hybrid breeding system through development of male sterility and fertility restorer lines. This helps in production of hybrids for increasing yields.




Global Status

The area under cultivation of GM crops has increased from 1.7 million hectares in 1996 to 189.8 million hectares in 2017 grown by over 17 million farmers, globally. While 24 countries planted commercialized biotech crops (16 GM crops) in 2017, an additional 43 countries have granted regulatory approvals for importing GM crops for use as food, feed and processing. The global status of cultivation of GM crops is given in Table 1 below:

Table 1: Status of cultivation of GM crops in various countries in 2017

S.No.	GM crops	Traits/Uses	Countries where approved
1	Alfalfa	Herbicide tolerance	USA
2	Apple	Anti-bruising and anti-browning	USA
3	Beet pepper	Virus Resistance	China
4	Canola	Herbicide tolerance and improved protection against weeds	Canada, USA, Australia, Chile
5	Carnation	Modified flower colour and herbicide tolerance	Australia, Columbia
6	Cotton	Improved insect protection, herbicide tolerance and improved protection against weeds	Australia, USA, China, Mexico, South Africa, China, Argentina, India, Columbia. Burkino Faso, Sudan, Pakistan, Brazil, Myanmar, Paraguay, Costa Rica
7	Egg Plant (Brinjal)	Insect resistance	Bangladesh
8	Maize	Improved insect protection and herbicide tolerance for efficient weed management.	Canada, USA, Argentina, Brazil, South Africa, Uruguay, Philippines, Chile, Columbia, Honduras, Spain, Portugal, Paraguay, Cuba, Czech Republic, Romania, Slovakia
9	Papaya	Virus resistance	USA, China

10	Petunia	Modified flower color	China
11	Poplar	Insect resistance	China
12	Potato	Improved quality, anti-bruising and anti-browning	USA
13	Soybean	Improved insect protection and herbicide tolerance for efficient weed management	USA, Argentina, Canada, Paraguay, Mexico, Bolivia, Brazil, Chile, South Africa, Romania, Uruguay, Costa Rica
14	Squash	Resistance against watermelon mosaic virus and zucchini yellow mosaic virus	USA
15	Sugar beet	Herbicide tolerance	USA and Canada
16	Tomato	Delayed Ripening, Virus resistance	China

Source: ISAAA Global Status of Commercialized Biotech/GM crops, 2017

In addition, beans, sugarcane and cowpeas have been approved by Brazil, Indonesia and Nigeria and are expected to be planted in the near future. Brazil has also approved GM Eucalyptus, an important pulp and paper producing tree.

Status in India

As indicated earlier, Bt cotton is the only GM crop approved for commercial cultivation in India as on date. The total area under Bt cotton has increased from 0.05 million hectares in 2002 to 11.4 million hectares in 2017. As of now, Bt cotton is cultivated in more than 90% of the area under cotton cultivation in India. In terms of area under cultivation under GM crops, India is fifth largest producer of cotton in the world after USA, Brazil, Argentina and Canada.

Several public and private sector institutions are involved in the research and development of GM crops in India (Table 2) . Field trials have been undertaken with more than 20 plants with varying traits such as hybrid seed production, insect resistance, herbicide tolerance etc.

Table 2: An indicative list of GM crops under research and development/ field trials in India

S. No.	Plant	Trait
1.	Banana	Antimicrobial peptide (AMP) gene
2.	Brinjal	Insect resistance
3.	Cabbage	Insect resistance
4.	Castor	Insect resistance
5.	Cauliflower	Insect resistance
6.	Chickpea	Abiotic stress tolerance, insect resistance
7.	Corn	Insect resistance, herbicide tolerance
8.	Cotton	Insect resistance, herbicide tolerance
9.	Groundnut	Virus resistance, abiotic stress tolerance
10.	Mustard	Hybrid seed production
11.	Okra	Insect resistance
12.	Papaya	Virus resistance
13.	Pigeonpea	Insect resistance
14.	Potato	Tuber sweetening, fungal resistance
15.	Rice	Insect resistance, diseases resistance, hybrid seed production, nutritional enhancement
16.	Rubber	Abiotic stress tolerance
17.	Sorghum	Insect resistance, abiotic stress tolerance
18.	Sugarcane	Insect resistance
19.	Tomato	Insect resistance, virus resistance, fruit ripening
20.	Watermelon	Virus resistance
21.	Wheat	Effect of mutant strains Azotobacter

According to a survey conducted by MoEFCC in 2014 under the UNEP-GEF supported Phase II Capacity Building Project on Biosafety, over 85 different plants species were identified as being used in experimental work, including plants used for food, feed, fiber, fuel and dietary or medicinal purpose.

Section 3: Safety Assessment of GM Crops

Concerns have been expressed about safety of GM crops since their introduction in the mid-1990s, primarily because of the perception that modern biotechnology tools such as genetic engineering lead to creation of new species. Safety concerns associated with the use of GM crops broadly relate to risk to human and animal health, and environment. These differ greatly depending on the gene-crop combination, and may include:

* Potential risk of introducing toxins, allergens and other anti-nutrition factors in foods
* Potential likelihood of transgenes escaping from cultivated crops into wild relatives
* Changes in weediness potential
* Interaction with non-target organisms
* Resistance/tolerance of target organisms

Systematic safety assessment methodologies are in place that have been agreed on years of consultations under the aegis of international organizations and agreements viz. Food and Agriculture Organization (FAO), World Health Organization (WHO), Codex Alimentarius Commission, Organisation for Economic Co-operation and Development (OECD) and Cartagena Protocol on Biosafety. The potential changes introduced using genetic engineering are assessed using comparative risk assessment approach The underline assumption of this comparative approach is that traditionally cultivated crops have a history of safe use and thus serves as the comparator. As a consequence, safety assessment process gives conclusion on whether or not the GM crops is as safe as its conventional non-GM counterpart.

Fig 3: Components of Safety Assessment of GM crops

Safety assessment studies required for commercial release of a GM crops comprise of food and feed safety assessment and the environmental risk assessment coupled with information through the molecular characterization of the GM crops and characterization of the expressed, transgenic proteins (Box 2).

Box 2: Broad Information Requirements for Safety Assessment of GM crops

Effect of Genetic Modification and Protein Characterization

- Description of the GM crops
- Description of the biology of the non-modified host plant
- Description of the donor organism
- Description of the genetic modification
- Inheritance and stability of inserted gene(s)
- Molecular characterization
- Function/ specificity/ mode-of-action of expressed protein
- Protein expression levels
- History of safe use and consumption

Food and Feed Safety

- Toxicity assessment by animal toxicity studies such as acute and sub-chronic studies
- Assessment of allergenicity by comparing amino acid sequence homology of the newly expressed protein.
- Heat stability and susceptibility of the expressed protein to pepsin digestion
- Compositional analysis by comparing changes in the level of key nutrients, natural toxicants or anti- nutrients, secondary metabolites, physiologically active (bioactive) substance etc
- Livestock feeding studies
- Effect of processing

Environmental Safety

- Confirmation of expression level of new proteins: Quantify the expression level of the gene product associated with each introduced trait
- Field trial locations and experimental designs
- Description of the phenotype of the transformed plant
- Plant growth and specific observations recorded during the field trials
- Changes in weediness and aggressiveness potential
- Susceptibility to diseases and pests.
- Impact on non-target and beneficial organisms like predators, soil micro flora etc
- Changes in gene flow pattern through pollen flow studies and crossability studies with sexually compatible relatives

Govt. of India is following a case by case safety assessment of GM crops. The information requirement and analysis by regulatory authorities depends on the development stage of a particular product. Data requirement may also vary depending on the crop specific trait and intended use.

GM crops are permitted to be grown only after they have passed safety assessments and are not likely to pose risks for human health and environment. The data requirements for safety assessment are extremely rigorous for GM crops and are defined by regulatory authorities. Developers of GM crops (both public and private sector) test their products according to regulatory requirements which include detailed documentation of testing. Regulatory authorities undertake thorough analysis of the data and the protocols used to ensure the validity of the results. Additional information and additional testing may be asked by the regulatory agencies, if the data is not sufficient. The GM crops and foods that are currently on the international market have all passed safety assessments conducted by national authorities.

Section 4: Regulations of GM Crops in India

All GMOs including GM crops are regulated in India under the **Rules for the Manufacture/ Use/ Import/Export and Storage of Hazardous Microorganisms, Genetically Engineered Organisms or Cells (Rules, 1989)**, notified under the Environment (Protection) Act, 1986. The Rules, 1989 are very broad in scope, essentially covering the entire spectrum of activities involving GMOs and products thereof including sale, storage, exportation, importation, production, manufacturing, packaging, etc. These rules are implemented by the Ministry of Environment, Forest & Climate Change (MoEFCC), the Department of Biotechnology (DBT), Ministry of Science & Technology, Government of India and State Governments. Six competent authorities, their composition and roles have been notified under the Rules, 1989. The function of these six competent authorities is given in Table 3.

Table 3: Competent Authorities as per Rules, 1989

Statutory Committee	Function	Housed at
rDNA Advisory Committee (RDAC)	Review developments in biotechnology and recommend appropriate safety regulations for recombinant DNA research, use and applications	Department of Biotechnology, Ministry of Science and Technology
Institutional Biosafety Committee (IBSC)	Responsible for ensuring adherence to safety guidelines for experimentation at designated location	All organizations engaged in activities involving GMOs
Review Committee on Genetic Manipulation (RCGM)	Review all ongoing rDNA projects and approve experiments falling in risk category III and above; also responsible for bringing out manuals of guidelines for conduct of GMO research and use	Department of Biotechnology, Ministry of Science and Technology
Genetic Engineering Appraisal Committee (GEAC)	Authorized to review, monitor and approve all activities including import, export, transport, manufacture, use or sale of GMOs and products thereof from environment angle	Ministry of Environment, Forest and Climate Change
State Biotechnology Coordination committee (SBCC)	Monitoring and supervision at state level	Concerned State Governments
District Level Committee (DLC)	Supervision and compliance at district level	

Various sub-committees and expert committees are set up by Review Committee on Genetic Manipulation (RCGM) and Genetic Engineering Appraisal Committee (GEAC) on a case by case basis and comprise of experts from various disciplines drawn from public sector institutions to prepare and review various guidelines and biosafety data. Central Compliance Committees (CCC) are also set up for monitoring of confined field trials on case by case basis. The process of seeking approval of confined field trials and environmental release of GM crops is given in Figure 4.

Rules, 1989 mandate that no person shall import, export, transport, manufacture, store, process, use or sell any GMOs, substances or cells except with the approval of GEAC. Further permission from GEAC has to be obtained for scale up activities including field trials or production facilities. Unintentional release of GMOs is also not allowed.

GEAC also have powers to revoke approvals in case of any new information on harmful effects of GMOs, damage to the environment as could not be envisaged when approval was given and/ or non-compliance of any conditions stipulated by GEAC.

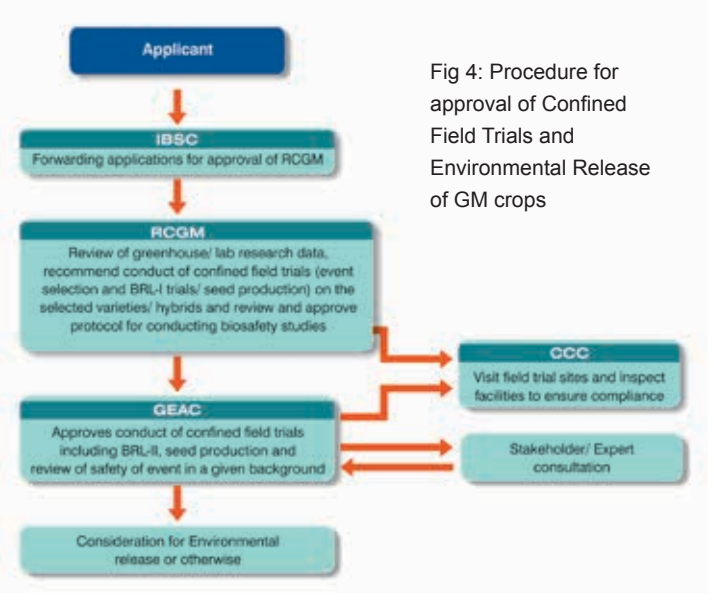


Fig 4: Procedure for approval of Confined Field Trials and Environmental Release of GM crops

Pursuant to above, GEAC adopted an Event Based Approval Mechanism (EBAM) for Bt Cotton Hybrids in 2008. As per the new procedure, Bt cotton hybrids expressing approved events and developed through conventional backcrossing are exempted from conduct of detailed biosafety studies as required for new events. All such cases are referred to a Standing Committee constituted by the GEAC. The new procedure lays down specific information to be submitted to the Standing Committee for taking a final view on the performance and suitability of a particular Bt Cotton hybrid/variety for a specific zone. Requirement for seeking approval from the State Government for the conduct of confined field trial has also been introduced by GEAC.

The GEAC has the authority to supervise the implementation of terms and conditions laid down in connection with the approvals accorded by it through the State Biotechnology Coordination committee (SBCC)/ District Level Committee (DLC)/State Pollution Control Board (SPCB) or any person authorised by the GEAC. If an order is not complied with, there is provision for imposing penalties including immediate intervention by the SBCC/ DLC in order to prevent damage to the environment, nature and health without issuing any orders at the expense of the person responsible for such damage. GEAC also has the authority to exempt an occupier handling a particular GMO from the provisions of Rules, 1989.

A **Gazette Notification No. GSR 584(E) to GSR 589(E)** dated 21st September, 2006 has been issued empowering Seed Inspectors/Seed Analysts/Laboratories notified under Seed Act, 1966 and Seed Control Order, 1983 under Environment (Protection) Act, 1986.

Supporting guidelines for GM crops

Rules, 1989 are implemented by six competent authorities through a series of biosafety guidelines. Guidelines have been issued for biosafety evaluation at each step of the development process of GM crops (Box 3).

Box 3: Biosafety Guidelines for GM crops in India

Contained Use

- Recombinant DNA Safety Guidelines, 1990 (Updated, 2017)
- Revised Guidelines for Research in Transgenic Plants, 1998

Confined Field Trials

- Guidelines for Conduct of Confined Field Trials of Regulated GE Plants, 2008
- Standard Operating Procedures (SOPs) for CFTs of Regulated GE Plants, 2008
- Guidelines for Monitoring of Confined Field Trials of Regulated GE Plants, 2008

Food Safety Assessment

- Guidelines for the Safety Assessment of Foods Derived from GE Plants, 2008 (Updated in 2012)
- Protocols for Food and Feed Safety Assessment of GE Crops, 2008

Environmental Safety Assessment

- Guidelines for Environmental Risk Assessment (ERA) of GE Plants, 2016
- Risk Analysis Framework, 2016
- ERA of GE Plants: A Guide for Stakeholders, 2016

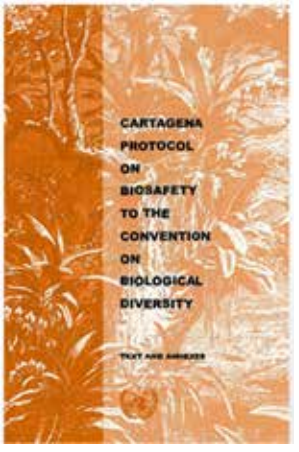
In addition to the Rules, 1989 provisions in other acts, rules and policies are also applicable to GM crops, which include:

Plant Quarantine (Regulation for Import into India) Order 2003, of the Ministry of Agriculture and Farmers Welfare covers regulation of import of germplasm/GMOs/ transgenic plant material for research purpose. National Bureau of Plant Genetic Resources (NBPGR) has been designated as the Competent Authority to issue import permits for import of seeds for research purposes after getting permission under Rules 1989 and to receive import material from customs authorities for quarantine inspection. The suppliers of the transgenic material are required to certify that the transgenic material has the same genes as described in the permit and that these transgenic materials do not contain any embryogenesis deactivator gene sequence.

National Seeds Policy (2002) of the Ministry of Agriculture and Farmers Welfare outlines that transgenic crops/varieties be tested to determine their agronomic value for at least two seasons by the Indian Council of Agriculture Research before any variety is commercially released in the market. The packages containing transgenic seeds/planting materialsshould carry a label indicating their transgenic nature including the agronomic/yield benefits, names of the transgenes and any relevant information.

The performance of the commercially releasedtransgenic varieties are monitored for at least 3 to 5 years by the Ministry of Agriculture and the State Departments of Agriculture.

Section 5: Cartagena Protocol on Biosafety



In addition to the national regulations, international agreements also impact the activities involving GM crops. The Cartagena Protocol on Biosafety (CPB) is a supplementary agreement to the Convention on Biological Diversity (CBD) that was adopted on 29 January, 2000 and entered into force on 11 September, 2003. As on March, 2019, 171 countries have ratified or acceded to the CPB. India is a Party to the CPB having ratified the Protocol on January 23, 2003. MoEFCC is the nodal ministry in India.

The CPB applies to transboundary movement, transit, handling and use of all living modified organism (LMOs) that include GM crops that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

LMOs covered under the CPB are categorized as under:

- LMOs for intentional introduction into the environment (seedlings, trees, animals for breeding, live fish, bacteria or other microorganisms)
- LMOs intended for direct use as food or feed, or for processing (e.g. agricultural commodities- corn, canola, cotton)
- LMOs for contained use (e.g. bacteria for laboratory scientific experiment)

Exemptions under the Protocol include LMOs that are pharmaceutical for humans if they are covered by other international agreements or arrangements and products derived from LMOs such as processed food (e.g. soybean oil, corn flour)

The CPB promotes biosafety by establishing practical rules and procedures for the safe transfer, handling and use of LMOs, with specific focus on regulating the transboundary movement of LMOs. There are 40 Articles in the CPB, which could be categorized into key elements and supporting tools and mechanisms. The four key elements include procedures for transboundary movement of LMOs, risk assessment and risk management, handling, transport, packaging and identification and information sharing as presented in Figure 5.

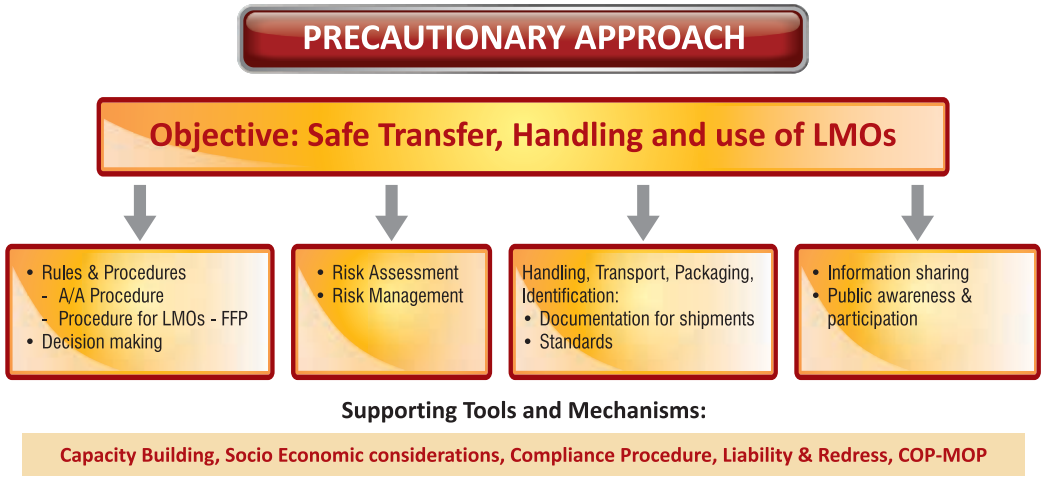


Fig 5: Key Elements of the Protocol

Specific procedures have been defined for LMOs for intentional introduction into the environment, that are subjected to Advanced Informed Agreement (AIA) procedures and LMOs for direct use as food, feed and processing (FFP) that may be subjected to a separate procedure. CPB requires decisions on import of LMOs for intentional introduction into the environment in accordance with scientifically sound risk assessment. Documentation requirements have been defined for various categories of LMOs, however all categories require reference to a unique identifier code, as indicated in the Box 4.

Box 4: LMO Unique Identifiers

- Documentation requirements for all categories of LMOs require reference to a unique identifier code. To date, only one unique identification system exists: OECD Unique Identifiers for Transgenic Plants
- OECD Unique Identifier is a simple alpha numeric code that is given to each living modified plant that is approved for commercial use
- Developers of transgenic plants are the ones to assign the unique identifier
- 9- digit code composed of 3 elements separated by dashes
  - 2 or 3 alpha numeric digits to designate the applicant;
  - 5 or 6 alpha numeric digits to design at the transformation event; and
  - 1 numerical digit for verification Example: MON-00810-6M on santo's YieldGardMaize
- Unique identifier codes can be used to search BCH for information about specific LMOs

LMO Quick-links are a tool developed to assist in the identification of LMOs in documentation accompanying their transboundary movement. LMO Quick-links are small image files, which can be easily copied and pasted in documentation accompanying LMOs for the purpose of providing information on a specific LMO. LMO Quick-links identify a LMO through the organism's unique identifier (for plants), trade name and a link to the BCH where more information on the LMO is available.

Biosafety Clearing House (BCH) is a website set up under the CPB to facilitate exchange of information on GMOs/LMOs by Parties. It is a repository of up-to-date information on LMOs and biosafety including information about the National laws, regulations, guidelines, competent national authorities and final decisions taken by countries that is Party. It is accessible at http://bch.cbd.int/

Information available on BCH is organized into National Records that are submitted by Parties and Reference Records that are submitted by general BCH users.

Icon	Approval of LMO for
	Intentional introduction into the environment
	Direct use as food
	Direct use as feed
	Processing
	Confined Use
	Pharmaceuticals
	Transit

Table 4: Icons used for conveying information about decisions

To facilitate easier understanding about results of queries involving decisions on LMOs, different icons have been used in the BCH as indicated in Table 4.

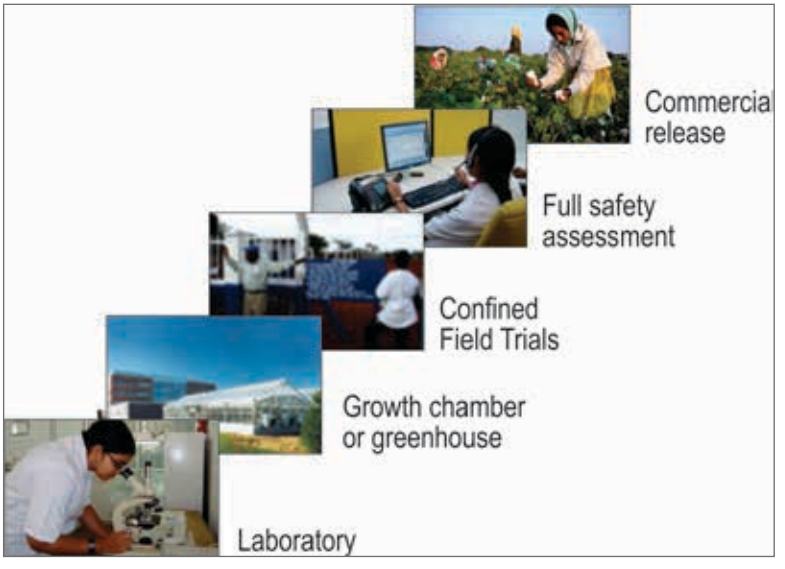
Other online databases which can be accessed for information on LMOs include OECD BioTrack Online Website (http://www2.oecd.org/biotech/); FAO GM Foods Platform (http://www.fao.org/food/food-safety-quality/gm-foods-platform/en/) and a website containing information about the Indian biosafety regulations, established by MoEFCC. It is accessible at http://geacindia.gov.in and provides information about decisions taken by the GEAC, the apex regulatory committee in India.

Section 6: Confined Field Trials of GM Crops

GM crops are required to go through field-testing so that they can be evaluated for their biosafety as well as agronomic performance before being released for use by the farmers. As GM crops may contain one or several additional genes than the conventionally bred crops, their field-testing is carried out under conditions to ensure that the materials tested remains within the trial site and hence, such trials are referred to as Confined Field Trials (CFTs). CFTs of GM crops are similar to field trials regularly done for conventional breeding, except that they are confined to the particular site.

CFTs are conducted with the objectives to test the efficacy of the inserted genes/introduced trait in the local environment under the real field conditions. Field trials enable selection of superior lines for development and facilitate the generation of safety data needed for subsequent risk assessment and approval. Conduct of CFTs is an essential step in the process of development and commercialization of a GM crops.

Information requirements for CFTs are very different from the commercial releases. The focus in CFTs is towards ensuring confinement measures, and not for elaborate safety data, as the required safety data is actually generated during the CFTs. On the other hand, full risk assessment is undertaken



prior to commercial releases. Therefore, separate questions, separate review and approval processes are followed in every country for the two steps.

In a CFT, biological and physical confinement measures are used to restrict GM crop material to a specific area of the environment and these research trials are thus considered globally as extensions of contained experimentation. CFTs are performed under stringent terms and conditions that confine the experimental material. It has been well established globally that CFTs can be performed safely and routinely by focusing on material and genetic confinement measures.

Types of CFTs generally conducted by developers of GM crops are as follows:

i. **Event Selection Trials:** Include planting small plots comprised of several to dozens of events of the same plant species for a preliminary evaluation to facilitate the selection of one to a few events for further evaluation.

ii. **Biosafety Research Level - I Trials (BRL-I):** Limited in size to no more than 1 acre (0.4 ha) per trial site location and a maximum cumulative total of 20 acres (8.1 ha) for all locations for each plant species/construct combination, per Applicant, per crop season.

iii. **Biosafety Research Level - II Trials (BRL-II):** Limited in size to no more than 2.5 acres (1 ha) per trial site location and number of locations to be decided on a case by case basis for each plant species/construct combination, per Applicant, per crop season.

iv. **Experimental Seed Production:** Production of seeds for the selected events under confined field trial conditions for the next phase of trials.

v. **Production of plant material for food and feed safety studies:** To generate plant material for undertaking various food and feed safety studies such as toxicity and feeding studies under confined field trial conditions.

vi. **Other environmental safety studies:** Trait or crop specific studies under confined field conditions for generating data on environmental safety e.g. residue analysis, crossability studies etc.

The regulatory steps followed for conduct of CFTs in India are described below:

- Based on information generated by the applicant in lab /greenhouse and on preliminary phenotypic evaluation of event selection, an application is made to IBSC for one to a few events for further evaluation.
- If recommended by IBSC the applicant may submit an application to RCGM for evaluation of the event along with experimental design, details of data to be generated, products from data generation.
- RCGM is the regulatory authority for BRL-I scientific evaluation trials. Being experimental trials, these trials are limited to no more than one acre per trial site location.
- GEAC is the regulatory authority for BRL-II scientific evaluation trials. These are generally limited in size to no more than 2.5 acres (1 ha/trial site location).
- The consent of the state governments is also taken, as per the recently issued directive.
- The number of locations is decided on case by case basis for each plant species/construct combination, per applicant per crop season.
- It may be noted that minimum of three seasons/ year's BRL scientific evaluation trials are required for consideration of an application to GEAC for release of an event, which generally consist of two years/seasons of BRL-I and one year/seasons of BRL-II scientific evaluation. The compliance of the regulatory conditions stipulated by the RCGM/GEAC for conduct of confined field trials of GM crops is monitored through Central Compliance Committees (CCC) consisting of subject-specific experts, representatives from RCGM/GEAC, representative from state agriculture department and state agricultural universities.

As mentioned in the **"Guidelines for the conduct of confined field trials of regulated genetically engineered plants, 2008",** the regulatory authorities i.e. RCGM and GEAC ensure that the CFTs are conducted keeping in view under conditions known to mitigate or prevent:

1. Pollen or seed mediated dissemination of the genetic entities of experimental crop plant;
2. Persistence of the GM crop or its progeny in the environment, and;
3. Introduction of the GM crop or its products into the human food or livestock feed pathways.

These measures are sufficiently described by the applicants in their application to RCGM and GEAC. Members of the committee carefully review the same and these measures then become the terms and conditions of permission letter (referred to as permit), if the application for field tests is approved. These terms and conditions also serve as a guide for the CCCs deputed by RCGM and GEAC for inspecting the field trial site and reviewing the compliance by the applicant.

Besides the requirement for complying with the guidelines and standard operating procedures (SOPs) for CFTs, the regulatory agencies prescribe the following for ensuring the confinement:

Fig.6 Spatial isolation

- The spatial isolation distance is the most commonly used method to restrict the pollen mediated gene flow Figure 6.
- On a case by case basis, the crop isolation distance may also be used in conjunction with other methods for ensuring reproductive isolation such as temporal isolation, pollen trap plant rows, bagging flowers, de-taselling, removing flowers before sexual maturity and terminated trial before onset of flowering. It may be noted that the risk of gene flow depends on the growth stage of a crop. It is a well established fact that there is no risk at vegetative stage, there is risk from pollen only at the flowering stage and risk from seeds at the stage of seed setting and crop maturity.
- Isolation distances prescribed by the regulatory authorities are based on accepted distance for pure seed production, which have been prescribed under the Indian Minimal Seed Certification Standards by Department of Agriculture and Cooperation, Ministry of Agriculture and Farmers Welfare. It may be noted that these standards are developed based on years of experimental data and have been accepted globally as standard methods for reproductive isolation by regulatory authorities.
- On a case-by-case basis, specific methods of physical confinement may also be advisable to prevent herbivore or the destruction of plant material by foraging animals, or the unauthorized harvest or removal of plant material by humans.

In addition to the above, the applicants are required to submit a validated event specific test protocol of 0.01% before undertaking the BRL-I trials, as per the directions of the Hon'ble

Supreme Court. An Officer in Charge of such experimental evaluation trials is responsible for conduct of each trial and the field trials are permitted only on the research farms of public and private research and development organizations or in their long leased land. All the plant material that is not retained for research purpose is destroyed by supervised incineration (Figure 7) after completion of the trial in the presence of officials from relevant state department of agriculture.

Fig 7: Destruction/Incineration of GM crop after completion of trial

The guidelines specify the post harvest monitoring for a specified period for each crop. During this period, the site cannot be used for planting the same or sexually compatible species as it is monitored for volunteers, which are rendered non-viable before flowering. Such post harvest restriction period varies for each crop depending on their biology.

While general principles and standard terms and conditions remain the same for all CFTs, specific conditions to be laid for each CFT vary according to the crop, the introduced trait and the locations/environment.

Section 7: Detection of GM Crops

The objective of this section is to introduce the methods used to detect presence of GM crop in a sample of seeds or plant material, to identify which GM crops are present and to calculate the quantity. In many instances, this work will not be done by frontline seed inspectors may be performed by seed testing or other laboratories.

Reasons for testing of GM crops in a sample include screening for the presence of GM crops, testing for specific GM crops and quantification of GM crops. For countries that have not approved the import of any GM crop, the detection of any GM content in a sample would mean that consignment is not allowed for sale or cultivation. Testing for specific GM crops is done to verify that the GM crops declared in the documentation accompanying the consignment is actually present. Another objective is to test for GM crops that have not been declared as being in the sample. This is important if a country has authorized some GM crops but not others and wants to make sure that unauthorized GM cropsare not cultivated in country. Testing for GM crops also used to calculate the quantity of GM content in a sample in the context of labeling.

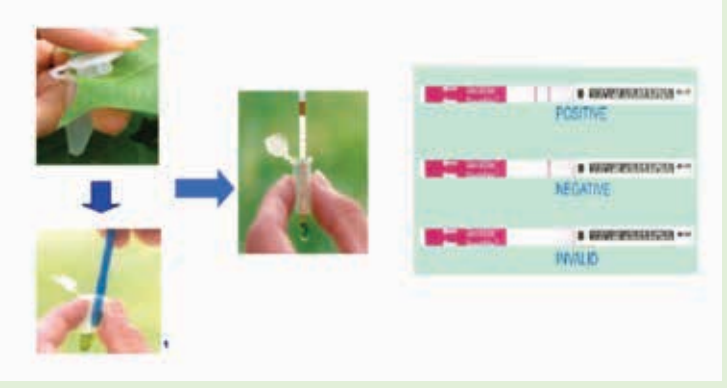
Methods for detecting GM crops: As indicated in Section 1, a GM crop is created by inserting a gene from one organism into the DNA of another organism and this new gene usually leads the organism to produce a protein that gives the organism a desired characteristics. In view of the above, there are two basic approaches to test the GM crops. These include protein based methods for testing for the proteins produced by the gene that has been inserted into the GM crop and DNA based testing for the introduced gene itself.

I. **Protein based methods:** These methods can be used for screening (yes/no) and quantification of expressed protein in a GM crop using strip test and Enzyme-linked immune sorbent assay (ELISA) based test respectively.

a **Strip tests** are simplest of all the detection methods. Strip test kits produced by different companies, include specially coated paper strips that are designed to detect specific proteins produced by different GM crops.

Methodology for Strip Test

Typically, a small sample is first ground into a powder. A liquid extraction buffer, included in the kit is added to a tube along with the powder. The tube is then shaken to allow the maximum amount of protein to be released into the buffer. A small amount of this mixture (referred to as extract) is transferred into vial. The coated paper strip is then placed in the vial. The result monitored as the colour of the strip changes indicating whether or not it is a GM crop. Unskilled personnel in the field can easily carry out strip based tests.



b. **ELISA based test:** This test uses antibody (polyclonal or monoclonal) raised against a specific protein encoded by transgene. These antibodies are colour coated to enable them to be easily detected and quantified. The kits for ELISA test are also produced by companies that specialize in GM crop testing. ELISA kits include plastic plates with number of wells, which are pre-treated so that the protein of interest in the sample will stick to the well.

Methodology for ELISA test

For the ELISA test, an extract is prepared by grinding the sample into a powder and adding an extraction buffer (similar to the process with strip tests). The extract is then added to the wells in a plate. If the extract contains the protein of interest then this protein will stick to the bottom and sides of the well.

Whether the protein has stuck to the well is not visible to the human eye so additional steps are needed to determine the results of the test. A reagent (a chemical used for analysis and reactions, also provided in the kit) is then added to the wells and it attaches to the protein of interest that is stuck

to the well. Finally, the results of the test are visualized with a colour development step. In this step, another chemical is added to the wells, which causes a reaction that changes the colour of the contents in the well. The darker the colour, the higher the concentration of the protein of interest. The Intensity of color is measure using an "ELISA Reader".

2. **DNA based testing:** The DNA that is introduced into an organisms to create a GM crop consists of several components and is known as a gene construct. Components of a gene construct are generally as follows:

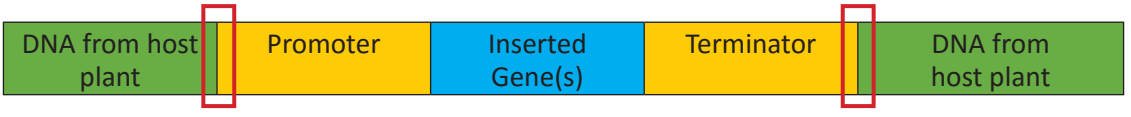
DNA from host plant	Promoter	Inserted Gene(s)	Terminator	DNA from host plant

DNA-based testing involves testing for any of the components of a gene construct.Some of the components e.g. promoters are widely used in development of different GM crops. In such cases detection of a promoter sequence helps in knowing presence of GM crops in a sample, but does not allow for specific identification of LMO.

It is possible to test for any one of the components of a gene construct in order to detect a GMO. A number of components, however, have been inserted into more than one GMO.For example, the 35S promoter isolated from the cauliflower mosaic virus (and thus known as the CaMV 35S promoter) is one of the most widely used promoters in living modified plants. It is used, for example, in both Liberty Link maize (product of Bayer Crop Science) and Roundup Ready cotton (product of Monsanto).

Using DNA-based testing to detect the CaMV 35S promoter will tell if the sample contains GMOs but it will not allow you to specifically identify which GMO has been detected. If a country has approved Liberty Link maize but has not approved Roundup Ready cotton then detecting the CaMV promoter that is common to both will not help in taking appropriate decisions.

A more specific test for detecting and identifying a particular LMO is to test for the combination of the host organism's DNA and either the promoter or the terminator from the gene construct. This is called 'Event-Specific detection'. Event-specific detection allows identification of the specific LMO in a sample.



DNA based testing involves multiplying/amplifying a specific DNA through polymerase chain reaction (PCR) technique. Specific gene/transgene/elements associated with the transgene can be amplified using a PCR machine. The amplified DNA is then visualized using the gel electrophoresis technique.

DNA methods are highly sensitive and can test for multiple GM varieties simultaneously. However, these requires highly skilled personnel, laboratory infrastructure and are more expensive. For both the protein and DNA based detection methods there are several general considerations that include sampling, food matrix effects on protein/DNA extraction, reference materials, method validation, harmonization of standards and access to information database.

In India, several public and private sector organizations have capabilities for detection of GM crops. There are also companies supplying various types of test kits. Four laboratories, strengthened under Phase II Capacity Building Project on Biosafety have been designated as the National Referral Laboratories to detect for the presence or absence of GM crops/GMOs under the Seeds Act, 1966.

The laboratories strengthened for detection of GM crops /GMOs include:

1. DNA Fingerprinting and Transgenic Crop Monitoring Lab (DFTCML), Department of Agriculture, Government of Andhra Pradesh, Guntur, Andhra Pradesh
2. ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi
3. Export Inspection Agency (EIA), Kochi Laboratory, Kochi; Kerala
4. Punjab Biotechnology Incubator (PBTI) Mohali, Punjab

More information about detection of GM crops can be seen at http://gmolabs.nbpgr.ernet.in:9090/ maintained by ICAR-NBPGR.

भारत का राजपत्र
The Gazette of India

असाधारण
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कृषि और किसान कल्याण मंत्रालय
(कृषि, सहकारिता एवं किसान कल्याण विभाग)
अधिसूचना
नई दिल्ली, 15 नवम्बर, 2017

2 THE GAZETTE OF INDIA EXTRAORDINARY [PART II—SEC. 3(ii)]

MINISTRY OF AGRICULTURE AND FARMERS WELFARE
(Department of Agriculture, Cooperation and Farmers Welfare)
NOTIFICATION
New Delhi, the 15th November, 2017

S.O. 3604(E).—In exercise of the powers conferred by sub-section (1) of Section 4 of the Seeds Act, 1966 (54 of 1966), read with clause (c) of Rule 5 of the Seeds Rules, 1968, the Central Government hereby declares the following laboratories as the National Referral Laboratories to detect the presence or absence of Living Modified Organisms and Genetically Modified Organisms under the said Act with effect from the date of publication, for the whole of India, namely:—

(i) DNA Fingerprinting and Transgenic Crop Monitoring Lab (DFTCML), Guntur (Andhra Pradesh);
(ii) ICAR-National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi;
(iii) Export Inspection Agency (EIA), Kochi Laboratory (Kerala);
(iv) Punjab Biotechnology Incubator (PBTI), Mohali (Punjab).

[F. No. 13-127/2017-SD-IV]

Section 8: Role of State Agencies in Monitoring of GM Crops

As indicated in section 3, the responsibility of monitoring the activities related to GMOs is vested with State Biotechnology Coordination Committee (SBCC) and District Level Committee (DLC). The SBCC in the states, shall have powers to inspect, investigate and take punitive action in case of violation of statutory provisions through the nodal department and the State Pollution Control Board/Directorate of Health/Medical Services. The DLC is to be set up in the districts wherever necessary under the District Collectors to monitor the safety regulations. The composition of the two committees is given in Box 5

Box 5 Composition of State Biotechnology Coordination Committee (SBCC) and District Level Committee (DLC)

SBCC

- Chief Secretary - Chairman
- Secretary, Department of Environment - Member Secretary
- Secretary, Department of Health - Member
- Secretary, Department of Agriculture - Member
- Secretary, Department of Industries and Commerce - Member
- Secretary, Department of Forests - Member
- Secretary, Department of Public works/ Chief Engineer, Department of Public Health Engineering - Member
- State microbiologists and Pathologists - Member
- Chairman of State Pollution Control Board

DLC

- District Collector - Chairman
- Factory Inspector - Member
- A representative of the Pollution Control Board - Member
- Chief Medical Officer (District Health Officer) – Member (Convenor)
- District Agricultural Officer - Member
- A representative of the Public Health Engineering Department - Member
- District Microbiologists pathologist (Technical expert) - Member
- Commissioner Municipal Corporation - Member

The Secretary, Department of Agriculture in the State is a member of SBCC and District Agriculture Officer is a member of DLC. State Agriculture Departments have a major responsibility towards monitoring compliance of conditions stipulated by GEAC for commercial release, field trials and seed production of GM Crops.

Further, the functionaries from State Agriculture Department implementing the Seed Act including seed laboratories and analyst have been empowered to take punitive action and the sampling procedures have been notified to ensure uniform action by the field staff. The State Agriculture Departments are also notified about the field trials by GEAC with copies of communications addressed to Secretary, Agriculture and Commissioner, Agriculture, simultaneously. In addition to the above, the State Agricultural Universities (SAUs) are also actively involved in monitoring of GM crops.

To summarize, State Agriculture Departments and State Agriculture Universities have an important role to play in the enforcement and monitoring of regulations regarding GM crops to harness their maximum benefit in a sustainable manner.

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