The development of a genetically engineered plant in research laboratories has to be progressively tested in contained facilities such as greenhouses and field conditions before its commercialisation. The information and data collected during these trials is critical for assessing agronomic performance as well as biosafety assessment required by the regulatory authorities.

From the regulatory standpoint, the terms and conditions governing CFTs include a combination of science based confinement measures together with a system of adequate monitoring.

This brochure provides an overview of the concepts and scientific principles of reproductive isolation and other effective risk mitigative measures adopted to minimize adverse impacts on the environment during CFTs. In addition the brochure emphasizes the importance of CFTs and also introduces the role that such trials play in both the product development pipeline and the regulatory review process.

This brochure is a part of **Biosafety Resource Kit** prepared under the UNEP-GEF Phase II Capacity Building Project on Biosafety being implemented by the Ministry of Environment, Forest and Climate Change.

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CONFINED FIELD TRIALS
OF GENETICALLY ENGINEERED PLANTS

Updated 2018

Phase-II Capacity Building Project on Biosafety

Ministry of Environment Forest and Climate Change

Ministry of Environment
Forest and Climate Change
Government of India

In association with

BCIL
Biotech Consortium India Limited
New Delhi

What are Confined Field Trials (CFTs) ?

Confined field trials (CFTs) are small-scale field experiments to address the biosafety requirements and evaluate the performance of specific trait(s) in genetically engineered (GE) plants.

These are similar to field experiments done for conventional breeding, but they are confined.

Why are CFTs necessary ?

Field experiments are an essential step in the development of crop varieties, regardless of whether these varieties are produced by conventional plant breeding or by advanced techniques of modern biotechnology, to determine the performance of new variety in realistic conditions over a range of locations and environments.

CFTs of GE plants are conducted to:

- Evaluate the expression of the engineered traits, their effectiveness under real field conditions and select the best performing lines
- Evaluate the plant characteristics (phenotype) and/or test the agronomic performance of the newly produced lines
- Collect biosafety data for presentation to regulatory authorities
- Produce plant material for further studies on food safety
- Incorporate, through conventional breeding the desired traits into local varieties which are better adapted to the local environmental conditions

Without a mechanism to conduct confined field trials, GE plants cannot be developed, adapted to local needs, and therefore cannot benefit farmers.

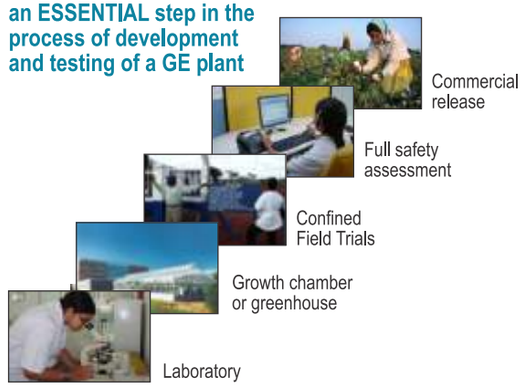
Key terms

1. GE plant: A genetically engineered (GE) plant is that in which the basic genetic material i.e. Deoxyribonucleic Acid (DNA) has been altered or modified using genetic engineering techniques to improve the attributes or make it perform new functions. GE plants are also referred as genetically modified (GM) plants, transgenic crops, or biotech crops.
2. DNA: DNA is the molecule that carries the genetic blueprint for life as it stores the genetic information and provides the key chemical information responsible for the inheritance of traits such as size, shape, color, build and other physical attributes of microorganisms, plants, animals and humans. All the living organisms can be modified because of the presence of DNA in every cell of all organisms.
3. Gene: A gene is a sequence of DNA that contains information that determines a particular characteristic/trait. Genes are units of inheritance that are passed from one generation to the next.
4. Event: A genotype produced from the transformation of a single plant species using a specific genetic construct.

Stage at which CFTs are conducted

- Scientists investigate potential beneficial traits, identify genes and carry out genetic transformations in research labs and green houses (contained conditions).
- For advancing research, CFTs are conducted in a real life environment.
- Safety assessment studies are undertaken for securing regulatory approval in the country where the plant will be grown, and/or its products consumed by humans or animals.
- Final step is commercial production.

Conduct of field trials is an ESSENTIAL step in the process of development and testing of a GE plant



Contained vs. Confined conditions

Contained conditions refer to work with GE organisms within contained facilities, such as a laboratory, a greenhouse, a nethouse and areas used for the storage and handling of experimental GE organisms. Under contained conditions there is a physical barrier or barriers to avoid direct contact of viable GE organisms with the environment.

Confined field trial is a limited field experiment of growing a regulated GE plant in the open environment under specified terms and conditions that are intended to mitigate the establishment and spread of the plant.

Can GE plants be scientifically evaluated in the greenhouse only?

- Data which fully represent the response of plants to the conditions likely to be encountered in a particular agro-ecological environment can be collected only by growing the plants outdoors in CFTs as it is virtually impossible to comprehensively replicate the outdoor environment in a greenhouse.
- Greenhouse studies are useful only in initial stages as these are conducted in controlled environment and are inadequate to predict how a plant will perform when grown outdoors under natural environmental conditions.
- Also greenhouse studies cannot be performed at a scale sufficient to comply with these regulatory requirements.

Without the field data, developers cannot make scientifically tenable predictions about the performance of the plants in the field or about the environmental safety of the plants.

Why are the field trials of GE plants "Confined" ?

- Field trials of GE plants are undertaken in confined conditions so as to minimize exposure since these products are still under safety evaluation as per regulatory requirements.
- Emphasis is on the implementation of the management practices designed to prevent exposure or escape of GE materials outside the trial site.

Types of CFTs

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 CFTs 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.

How to confine a field trial (Guiding Principles)?

Confinement of field trials is accomplished through appropriate management measures to:

- ensure the effective confinement of the experimental GE material so that it is not eaten by humans or livestock (Material Confinement)
- ensure reproductive isolation to prevent plants from pollinating compatible species and producing seed that escapes from the trial site (Genetic Confinement)
- prevent the persistence of the GE material in the environment by ensuring that the GE materials and any volunteers arising from the trial are completely destroyed (Post Harvest Land Use Restrictions)

Measures for confinement may vary according to the crop plant, the introduced trait and the environment

I Material Confinement

- Objective: To maintain control of the GE plant material at all times to prevent eating by humans or livestock and mixing it with non-GE material.
- Measures undertaken to ensure material confinement include:
  - (i) Appropriate packaging and labelling of GE plant material for transport to and from the trial site as per SOPs, secured storage, and measures for cleaning and/or disposing of the packaging material.
  - (ii) Cleaning of all equipments used for various activities during the trial such as planting, sampling, spraying, harvesting etc. so that no propagative plant material remains viable.
  - (iii) All the plant material generated during the trial (except that authorized by regulatory authorities to be retained for safety studies/ further research), to be disposed off by incineration, burying on the trial site, crushing or chemical treatment. No progeny can be retained for future planting without prior written authorization from the regulatory authorities.
  - (iv) To ensure security of the trial site to prevent incursion of humans or animals, measures include fencing, security guards, lockable gates etc. on a case by case basis.

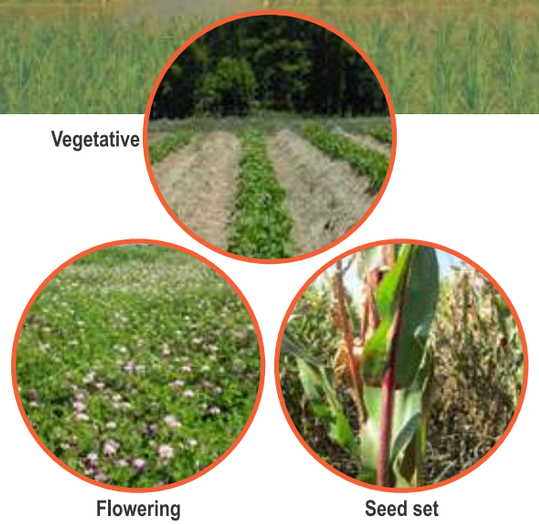
FIELD DEMARCATION

DESTRUCTION/INCINERATION

II Genetic Confinement

- Objective : To prevent any pollen-mediated gene flow from the trial sites.
- Measures : Different methods are used in CFTs to achieve genetic confinement also referred to as reproductive isolation. It involves use of barriers of various types to prevent hybridization between two plants that would otherwise be sexually compatible. Effective methods of reproductive isolation are determined by the reproductive biology of the plant species, and their application is generally crop-specific. Not all trials use every method. These methods help to prevent pollen dispersal or prevent fertilization to ensure genetic confinement. Some of the commonly used reproductive isolation methods are:

SPATIAL ISOLATION | TEMPORAL ISOLATION | REMOVAL OF FLORAL STRUCTURES | BAGGING REPRODUCTIVE PLANT PARTS | POLLEN TRAP ROWS | EARLY TERMINATION | ARTIFICIAL BARRIERS



Understanding biological basis of Pollen Flow

- The crop and recipient plants must be sexually compatible under natural conditions
- They must be externally pollinated (by wind or live vectors)
- They must be in proximity to each other
- They must flower at the same time
- The progeny must be able to survive, reproduce and produce fertile progeny
- Also the risk of gene flow depends on growth stage of a plant. There is no risk at vegetative stage vs. high risk from pollen at flowering stage and high risk from seeds at seed set and crop maturity stage.

Spatial Isolation

- Spatial isolation by maintaining a minimum isolation distance is the most commonly used method to restrict the pollen-mediated gene flow. On a case by case basis, it may also be used in conjunction with other methods such as temporal isolation, pollen trap plant rows, bagging flowers, detaselling, removing flowers before sexual maturity and termination of trial before onset of flowering.
- Isolation distances prescribed by the regulatory authorities are based on accepted isolation 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.
- These standards are developed based on years of experimental data and have been accepted globally as standard methods for reproductive isolation by regulatory authorities.

Minimum isolation distances for crops prescribed in India under field trials

Crop	Minimum isolation distance
Cotton	50 m
Maize	200 m
Rice	10 m
Brinjal	200 m
Okra	250 m

Source: http://agricoop.nic.in/imagedefault/seed/INDIAN_MINIMUM_SEED_CERTIFICATION_STANDARDS.pdf

Bagging Reproductive Plant Parts

- Field trials of some species can be isolated from same or related species by bagging the flowers or inflorescences to ensure that there is no pollen release before anthesis (the time of flowering or pollination).
- The inflorescences must remain bagged until no more pollen shedding occurs.

Temporal Isolation

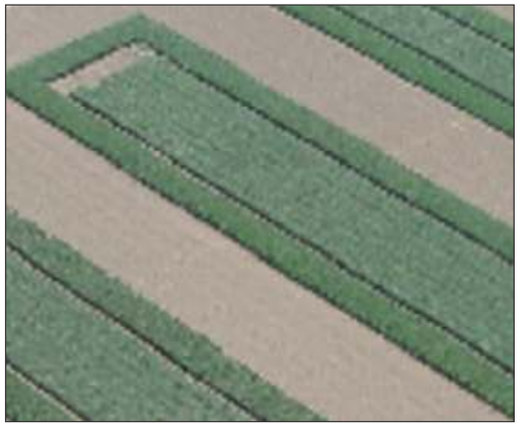
- Reproductive isolation can also be achieved by temporal isolation, which requires staggering the planting of the field trial so that pollen shedding is complete before any other pollen is shed by plants of the same species or sexually compatible species that may be cultivated within the reproductive isolation distance.
- This method of reproductive isolation must be used with caution as accurate prediction of time of anthesis may be difficult due to inherent variability in growing conditions.
- Close monitoring must be carried out to ensure that anthesis by the GE plants is non-concurrent with that of other relatives or plants of the same species within the isolation distance.

Removal of Floral Structures

- Removal of flowers from the regulated GE plants prior to the pollen shed is also used for reproductive isolation in CFTs.
- As with temporal isolation, timely removal of flowers require a rigorous monitoring to ensure that all the flowers are removed before anthesis.
- One of the most common examples regarding the removal of floral structure in CFTs is detasseling in case of maize. Detasseling is done by removing the pollen producing flowers and the tassel from the top of maize plants. It is employed for pollination control to cross-breed, or hybridize, two varieties of corn.

Pollen Trap Rows

- Field trials of some GE plants may be reproductively isolated from the same or related species by planting uninterrupted perimeter border of the conventional plant species referred to as pollen trap rows or border rows or guard rows.
- This method is particularly useful when the GE plants are tested for insect resistance, as then the border or guard rows can attract the insects and reduce the flow of pollen via insects. To ensure the above, the plants planted as border rows must flower at the same time as the GE plants and be of the same growth habit and height.
- Typically the variety used to plant the border rows should be the same or similar genotype planted at the same time as the GE plant and at a density similar to the GE plants in the trial site.
- The width of the border row is species-specific and should be a continuous row without any break in the perimeter.



- Close monitoring must be carried out to ensure that the border plants do not flower earlier but flower concurrently as the experimental plants.
- The use of border rows can pose management challenges such as movement of field equipment through the rows and remediation if flowering of the border variety is asynchronous with the trial plant.

Early Termination

- Early termination, including destruction of the trial plants prior to anthesis and pollen shed is a method of reproductive isolation that can be generally applied for all plant species, if it is compatible with the experimental objectives.
- The growth of the GE plants need to be carefully monitored to determine when to terminate the trial, as unexpected environmental conditions could accelerate the growth of the plants or flowering.

Artificial Barriers

- Reproductive isolation may also be achieved by placing physical barriers such as screening material around the trial plants, also referred to as tenting. Such material should be of a mesh size sufficient to prevent the transfer of pollen.

III Post-Harvest Land Use Restrictions

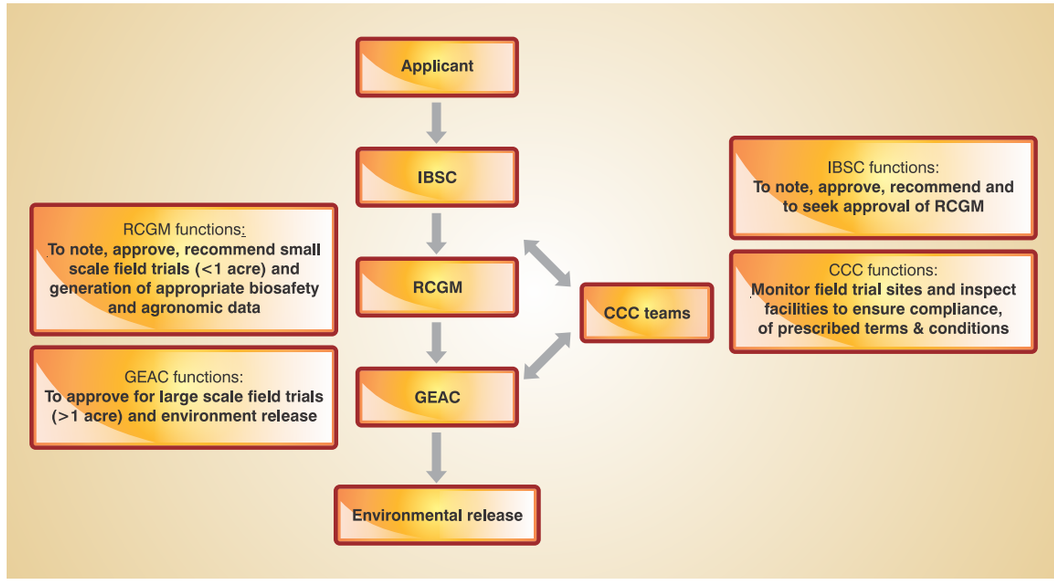
- **Objective :** To prevent persistence by maintaining control of CFT sites in the following years/season by eliminating volunteers.
- **Measures :** Progeny arising from the GE plants at the field trial site are known as 'volunteers', and must be prevented from establishing and flowering after termination of the trial. Depending on the nature of the propagative material remaining in the trial site and the biology of the crop plant, a period of post-harvest restriction and monitoring is prescribed by the regulatory authorities. The precautions to be implemented during post-harvest period include:
  - ❖ Area under restriction must be monitored during the post-harvest period to ensure that any prohibited plants (volunteers or sexually compatible species) are destroyed prior to flowering.
  - ❖ No plants of the same or sexually compatible species may be planted in the restricted area during this period.
  - ❖ Land use of the restricted area must be compatible with requirements for monitoring and removal of prohibited plants.
- The post-harvest restriction period begins immediately upon final harvest or termination of the CFT at the trial site.
- Monitoring for and destruction of prohibited plants also applies to the isolation distance around the trial site if reproductive isolation was breached during the trial.
- If any prohibited plants are permitted to flower, the post-harvest restriction period will be extended by an additional term equal to the original post-harvest restriction period.

Monitoring for Volunteer Crop

Regulation of CFTs of GE Plants in India

CFTs of GE plants are regulated in India as per the Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms, Genetically Engineered Organisms or Cells, 1989 (Rules, 1989) notified under the Environment (Protection) Act, 1986. These rules are implemented by the Ministry of Environment, Forest & Climate Change, Department of Biotechnology and State Governments through various committees. The Government of India is following a policy of case by case approval of CFTs of GE plants. The regulatory steps followed for approvals are as follows:

- The initial assessment of an application for CFT begins at the institutional level itself.
- Application to Institutional Biosafety Committee (IBSC) is based on information generated by the applicant in lab/greenhouse and on preliminary phenotypic evaluation.
- If recommended by IBSC, an application in the prescribed format along with information on experimental design and details of data to be generated is submitted to Review Committee on Genetic Manipulation (RCGM).
- RCGM is the regulatory authority for Biosafety Research Level I (BRL - I) trials (size limited to no more than one acre per trial site location).
- Genetic Engineering Appraisal Committee (GEAC) is the regulatory authority for Biosafety Research Level II (BRL - II) trials (size are generally limited to no more than 2.5 acres/1 ha/trial site location).
- The number of locations for BRL-II trials is decided on case by case basis for each plant species/gene combination, per applicant per crop season.
- No person can establish a CFT of any GE plant in India without the prior approval of RCGM and GEAC under Rules, 1989.
- Minimum of three seasons/ year's CFTs are required for consideration of an application by GEAC for release of an event, which generally consist of two years/seasons of BRL-I and one year/season of BRL-II scientific evaluation.
- All CFTs are monitored by Central Compliance Committees (CCCs).



Selection of Trial Sites

CFTs are usually carried out on a small scale, often on not more than one hectare area, at experimental stations such as those under the control of National Agricultural Research Systems (NARS), local universities, or private sector research units. In case where they have to be conducted in a farmer's field, the land has to be leased for sufficient duration of time including the post-harvest restriction period. The organizations conducting field trials have to be staffed by competent scientists with sound experience in the safe conduct of field trials and have capacity to evaluate the performance of new varieties for farmers. Regulatory authorities evaluate the suitability of the trial sites proposed by applicants taking into consideration the location, presence of sexually compatible species, proximity to protected areas, endangered/protected species etc.

Size and Number of Confined Field Trials

In order to maintain the integrity of the review and approval system, and to ensure adherence to the regulatory requirements, the Regulatory Authorities may restrict the number of confined field trial applications or approvals granted, and/or the size of authorized trials. These restrictions shall be determined by specific circumstances, and may be applied with respect to genetic constructs, phenotypic traits, field sites or other criteria.

Safety Assessment Requirements Prior to Conduct of CFTs

It is a common misunderstanding that confined field trials should be subject to essentially the same risk assessment process as for commercial releases. A detailed risk assessment is more correctly applied to the environmental release of GE plants for unconfined or commercial cultivation and not for field trials as the very purpose of conduct of field trials is to test efficacy and safety of a GE plant in real life environment. GE plant development cannot advance past the laboratory stage unless regulatory systems permit the confined field evaluation of GE plants.

Terms and Conditions Specified for CFTs

CFTs are performed under stringent terms and conditions that confine the experimental material. 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.

Confinement 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. Key terms and conditions applicable for all trials are as follows:

- All CFTs of GE plants are to be conducted in accordance with the "Guidelines and Standard Operating Procedures for CFTs of regulated, GE plants, 2008".
- The isolation distance is the most commonly used method for reproductive isolation. On a case by case basis, other methods such as temporal isolation, pollen trap plant rows, bagging flowers, detaselling etc. may be prescribed in conjunction with isolation distance.
- No seed or other plant materials from the CFT may enter the human food or animal feed chains.
- A Trial-in-Charge is to be designated for each trial site, who would be responsible for conduct of the trial
- All the plant material that is not retained for research purpose is destroyed by supervised incineration after completion of the trial.
- A notice board indicating the purpose and duration of the CFT along with other key details is to be mounted at the trial site.
- Adequate records of all activities including trial site compliance, transportation, storage, management, harvest/disposition and post-harvest monitoring are to be maintained by Permitted Parties.
- In the event of any accidental or unauthorized escape of GE plant material, the regulatory autho-rities are to be immediately informed, positively within 24 hours.

Monitoring/ Inspection of CFTs

- As the trial is done with plants that are 'regulated', or not yet approved for general release, the Regulatory Authority maintains oversight of the trial, through periodic inspections on the progress and compliance of the trial.
- Each field trial is monitored by a CCC constituted for a specific authorization on a case by case basis.
- The CCC consists of subject-specific experts, representatives from RCGM/ GEAC, representative from state agriculture department and state agricultural universities.
- Monitoring is undertaken at various stages during the conduct of confined field trials. These include pre-sowing, sowing, and various stages of crop development, harvest and during the period of post-harvest land use restriction.
- Monitoring agencies also have the authority to investigate contained facilities that may be used for the storage of regulated GE plant material.

Crop Specific Biology Documents

Crop specific biology documents are useful reference for conducting CFTs and safety assessment. Biology documents provide an overview of pertinent biological information on the untransformed (i.e., conventional or non-transgenic) species as a comparator against which GE plants are evaluated during the safety assessment process. These documents define the baseline information which serves as a resource tool for planning of CFTs by the developers, researchers and regulatory agencies.

MoEFCC and DBT prepared five biology documents on cotton, okra, rice, maize and brinjal in 2008. Eight additional biology documents on Sorghum, Mustard, Potato, Papaya, Chickpea, Pigeonpea, Tomato and Rubber in 2016.

Guidelines and SOPs for CFTs of Regulated GE Plants in India

Guidelines and Standard Operating Procedures for the conduct of CFTs of regulated, GE plants in India have been prescribed by the regulatory agencies under Rules, 1989. These guidelines summarize the information requirements and procedures used by the regulatory committees for evaluating and approving applications for confined field trials. The guidelines consist of the following:

- Guidelines for the Conduct of Confined Field Trials of Regulated, GE Plants
- Application Form for Confined Field Trials
- Standard Operating Procedures (SOPs) for Confined Field Trials of Regulated, GE Plants
- Recording Formats
- Guidelines for the Monitoring of Confined Field Trials of Regulated, GE Plants
- Comprehensive Glossary of Terms

E-learning Course on Compliance Management of Confined Field Trials

An e-learning course on "Guidelines & Standard Operating Procedures (SOPs) for Confined Field Trials of Regulated, GE Plants" has been prepared by Biotech Consortium India Ltd. (BCIL) and the South Asia Biosafety Programme (SABP) with an objective to strengthen the management and monitoring of CFTs of GE plants. This is an useful tool for the Trial-in-Charge and all those engaged in the conduct and monitoring of CFTs including members of the various committees associated with approval and/or monitoring of CFTs, scientists from public and private sector engaged in research on GE plants, and other interested stakeholders including students. The course can be accessed at the link: http://cft.biotech.co.in