Journal of Scientific & Industrial Research
Vol. 59, February 2000, pp 114-120

# Indian Rules, Regulations and Procedures for Handling Transgenic Plants*

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The Indian Government had promulgated the Rules for dealing with genetically modified organisms (GMOs) in 1989. The GMOs include the products of GMOs as well. The Rules define the authorities. They also elaborate the implementation structures for conducting research and for the commercial applications of GMOs. The major Indian developments in transgenic plants include work on transgenic cotton, Indian mustard/oil seed rape, tomato, brinjal, cauliflower, cabbage, potato, tobacco, chili, bell pepper, and rice. The research on GMOs is monitored by the Review Committee on Genetic Manipulation (RCGM) which stewards the generation of bio-safety data starting form lab to contained green house experiments to small-scale contained field trials. RCGM also authorizes the import/export/transfer of transgenic genetic materials for research use only. The bio-safety data required to be generated include: rationale for the development of GMOs, details of their molecular biology, comparison of germination rates, phenotypic characteristics, study of gene flow, invasiveness and weed formation possibilities, susceptibility to diseases and pests, effect on non-target organisms and food safety evaluation. The agronomic evaluation is also conducted. The Genetic Engineering Approval Committee provides authorization of GMOs for commercial release based on Bio-safety evaluation data.

## Introduction

In order to protect the environment the Union Government of India enacted[1] the Environment (Protection) Act (EPA) in 1986. The Act was to be the instrument for preserving the environment and to minimize the risks from pollutants and contaminants that can affect the flora and fauna, taking also into account the risks to human and animal health. While it was the expectation that Genetically Modified Organisms (GMOs) were going to play an important role in the economic upliftment of the country in its various facets including agriculture, human and animal health care system, industrial products and environment management, concurrently it was also realised that there could be unintended hazards and risks from the use of GMOs and products thereof, if the new technology was not properly assessed and utilized. Keeping these in view the Indian Government had issued Rules and Procedures (Rules) for handling GMOs and hazardous organisms through a Gazette Notification No. GSR 1037(E) dated 05 December 1989 from the Union Ministry of Environment and Forests[2]. The Rules cover all kinds of GMOs and products thereof, which are controlled commodities for handling and use in the country under the EPA.

*Views expressed in the paper are those of the authors and they do not necessarily express the views of the organisation to which the authors belong

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## Structure for Implementation of Rules for dealing with GMOs

The Rules clearly define Competent Authorities and structured compositions of such Authorities for the handling of all aspects of GMOs and products thereof, in India. Presently, there are six competent authorities as stated below, indicating their broad responsibilities and authorities too:

### *(i) The Recombinant DNA Advisory Committee (RDAC)*

This Committee constituted by the Department of Biotechnology of the Union Ministry of Science and Technology is to monitor on the developments in biotechnology at national and international levels. The RDAC submits recommendations from time-to-time that are suitable for implementation for upholding the safety regulations in research and applications of GMOs and products thereof. This Committee prepared the first Indian Recombinant DNA Biosafety Guidelines in 1990, which was adopted by the Government for conducting research and handling of GMOs in India[3].

### *(ii) The Review Committee on Genetic Manipulation (RCGM)*

The RCGM is constituted by the Department of Biotechnology to monitor the safety aspects of ongoing research projects and activities involving genetically engineered organisms. The Committee is also mandated to bring out Manuals of Guidelines specifying procedures for regulatory process with respect to activities involving genetically engineered organisms in research, use and applications including industry, with a view to ensure environmental safety. All ongoing projects involving high risk category and controlled field experiments shall be reviewed by the RCGM to ensure that adequate precaution and containment conditions are followed. The RCGM can lay down procedures restricting or prohibiting production, sale, importation, and use of GMOs.

RCGM can approve applications for generating research information on transgenic plants. Such information may be authorized to be generated in contained green house as well as in small plots. The small experimental field trials are limited to a total area of 20 acres in multi-locations in one crop season. In one location where the experiment is conducted with transgenic plants, the land used should not be more than one acre. The design of the trial experiments requires the approval of the RCGM. The design of the experimental plot in open environment is made to seek answers to relevant and necessary questions on environmental hazards including risks to animal and human health. Data are required to be generated on economic advantage of the transgenics over the existing non-transgenic varieties. RCGM can also direct the generation of toxicity, allergenicity and any other relevant data on transgenic materials in appropriate systems.

The RCGM can issue clearances for import/export of etiologic agents and vectors, transgenic germplasms including transformed calli, seed and plant parts for research use only. All experiments using GMOs which belong to category III risks and above as elaborated in the guidelines require a permit to be issued by the Department of Biotechnology for authorizing such experiments. All such permits are issued on the basis of the recommendations of the RCGM. According to the Indian classification of risks, the category-I risks involve routine recombinant DNA experiments in lab and work involving defined genes/DNA of microbial, plants or animal origin which are generally considered as safe. Category-II risks involve lab and contained greenhouse experiments involving genes or DNA of microbial, plant or animal origin which are non-pathogenic to human, but can have implications on plants and insects. Category- III involves genes/DNA of microbial, plant or animal origin which can cause alterations in the biosphere and does not fall in category-I and II. All open field experiments of GMOs are considered to belong to category-III risks.

The RCGM can put such conditions as would be required to generate long-term environmental safety data from the applicants seeking release of transgenic plants into the open environment.

The RCGM had revised the 1990 Guidelines for conducting research using GMOs in May, 1994 (ref. 4) and subsequently in August, 1998 (incorporating further amendments in September, 1999) (ref. 5). The guidelines capture detailed procedures for conducting contained field experiments using GMOs; they also provide guidance for generating food safety data for transgenic plants. Contained field experiments are designed to account for and to arrest the escape of the transgenic plants or plant parts or seeds set in the plants into the open environment; they are also designed to create reasonably effective barrier to prevent the escape of the transgenic pollen into the open environment.

*(iii) Institutional Biosafety Committee (IBSC)*

This Committee is constituted by organizations involved in research with GMOs. The Committee requires the approval of the Department of Biotechnology. IBSC also has a nominee from the Department of Biotechnology who oversees the activities to ensure that safety aspects in accordance with the safety guidelines are fully adhered to by the organization. Every R&D project using GMOs has to have an identified investigator who is required to inform the IBSC about the status and results of the experiments being conducted. Experiments belonging to Category I and II risks, as well as all experiments conducted with GMOs in the contained lab and contained green house conditions can be approved by the IBSC, however the synopsis of all such experiments is required to be reported to the RCGM in the Department of Biotechnology in the form of reports from time-to-time in a prescribed format. Such information along with the progress of research work is also required to be reported to the RCGM as a mandate at least once in six months.

*(iv) Genetic Engineering Approval Committee (GEAC)*

This Committee functions as a body in the Ministry of Environment and Forests and is responsible for approval of activities involving large scale use of GMOs in research, industrial production and applications. The clearance of GEAC is only from environmental angle under the EPA. All other relevant laws would apply even though EPA clearance is available for using GMOs and products thereof; e.g., drugs made through GMOs would require separate approval for manufacture and use under the Indian Drugs Act; production of GMOs is also authorized under Indian Industries (Development and Regulation) Act, and therefore these clearances are also mandatory.

Large scale experiments beyond the limits specified within the authority of RCGM are authorized by GEAC only. The GEAC can authorize approvals and prohibition of any GMOs for import, export, transport, manufacture, processing, use or sale under Rule 7,8,9,10 and 11. All such authorizations are usually conditional, and such conditions are guided by Rule 13.

*(v) State Biotechnology Coordination Committee (SBCC)*

This Committee, headed by the Chief Secretary of the State is constituted in each Indian state where research and applications of GMOs are contemplated. The Committee has the powers to inspect, investigate and take punitive actions in case of violations of the statutory provisions. The Committee coordinates the activities related to GMOs in the State with the Central Ministries. This Committee also nominates State Government representatives in the activities requiring field inspection of activities concerning GMOs.

*(vi) District Level Committee (DLC)*

This Committee constituted at the district level is considered to be the smallest authoritative unit to monitor the safety regulations in installations engaged in the use of GMOs in research and applications. The District Collector heads the Committee who can induct representatives from State agencies to enable the smooth functioning and inspection of the installations with a view to ensure the implementation of safety guidelines while handling GMOs, under the Indian EPA.

**Indian Scenario on Transgenic Plant Research**

The first transgenic plant experiment in the field was started in 1995 when *Brassica juncea* plant containing *Bar* gene regulated with plant specific constitutive promoters and linked with *Barnase* and *Barstar* genes regulated with floral tissue specific promoters were planted at Gurgaon (Haryana), India, under contained conditions. These studies were conducted to assess the extent of pollen escape[6]. Subsequently, several experiments have been started in the field in different locations using transgenic mustard, cotton and tomato. Several Indian institutes and organisations have claimed to develop transgenic plants, which are ready for green house/poly-house evaluation and some are ready for field evaluation as well. Table 1 gives a list of the major Indian developments reported so far in transgenic plants.

**Conditions for Trials Using Transgenic Plants**

The RCGM monitors research on transgenic organisms in the laboratory, in the contained green house and in contained open fields. In the field under contained conditions, the agronomic advantages of the transgenic plants are also assessed. The RCGM looks for information on environmental safety including human and animal food safety issues. The information sought from the trials of GMOs is summarized briefly in Table 2 (ref. 5).

The genetic materials can be allowed to be imported/exported/transferred by the RCGM for research use only, based on applications submitted through the IBSC.

For conducting experiments in contained greenhouse, designs have been worked out for constructing low cost but substantially contained environment where temperature, light and humidity can be controlled to a considerable extent. Nets have been recommended that arrest the entry/exit of insects below 0.3 mm diam[5].

The issues that are taken into consideration before authorizing field trials under contained conditions include the potential of the transgenic plants for dissemination into the open environment such as through cross-pollination, the dispersal mechanism of the pollens as well as the seeds, the presence of wild members of the species in the eco- system and the presence of other non-transgenic planting materials in the vicinity. While designing field experiments efforts are made to maintain appropriate reproductive isolation so as to prevent the likely-hood of seed setting outside the experimental plot. The transgenic plants are isolated from the gene pool represented by sexually compatible plants to prevent the escape of transgenes. Conditions are also introduced in certain cases to prevent flowering of plants. It is ensured that the genes or the genetically modified plants are not released into the environment beyond the experimental sites. Only such plants are taken into the open environment for experimentation which have the minimum chance of unintended and uncontrolled adverse affects. The time of sowing, flowering, and planting are also taken note of. Only those plants have been used in Indian trials for open field experiments under contained conditions, where the transgenes are considered to be safe or where the pollens are linked with imparting male sterility properties. Experiments have

Table 1 — Indian developments in transgenic research and applications

| Institute | Plants/crops used for transformation | Transgenes inserted | Aim of the project and progress made |
|---|---|---|---|
| Central Tobacco Research Institute, Rajahmundri | Tobacco | Bt toxin gene Cry1A(b) and Cry1C | To generate plants resistant to *H.armigera* and *S.litura*. One round contained field trial completed. Further evaluation under progress |
| Bose Institute, Calcutta | Rice | Bt toxin genes | To generate plants resistant to lepidopteran pests. Ready to undertake Green House testing |
| Tamilnadu Agricultural University, Coimbatore | Rice | Reporter genes like *hph* or *gus A* | To study extent of transformation |
| Delhi University, South Campus, New Delhi | Mustard/rape seed | *Bar, Barnase, Barstar* | Plant transformations completed and ready for green house experiments |
| | Rice | Selectable marker genes, e.g., *hygromycin* resistance and *gus* | Gene regulation studies. Transformations completed. |
| Indian Agricultural Research Institute Sub-station, Shillong | Rice | Bt toxin gene | To impart lepidopteran resistance, transformations in progress |
| Central Potato Research Institute, Simla | Potato | Bt toxin Gene | To generate plants resistant to lepidopteran pests. Ready to undertake Green House trials |
| M/s Proagro PGS (India) Ltd, New Delhi | Brassica/mustard | *Barstar, Barnase, Bar* | To develop better hybrid cultivars suitable for local conditions: over 15 locations contained field trials completed. Further contained open-field research trials in progress at multi-locations |
| | Tomato | *Cry*1A(b) | To develop plants resistant to lepidopteran pests; glass house experiments and one season contained field experiment completed. Further experiments in progress |
| | Brinjal | *Cry*1A(b) | To develop plants resistant to lepidopteran pests; glass house experiments in progress |
| | Cauliflower | *Barnase, Barstar* and *Bar* | To develop hybrid cultivars for loacal use; glass-house experiments in progress |
| | Cauliflower | *Cry*1H/*Cry* 9C | To develop resistance to pests; glass house experiments in progress |
| | Cabbage | *Cry*1H/Cry9C | To develop resistance to pests; glass house experiments in progress |
| M/s MAHYCO, Mumbai | Cotton | *Cry1*A(c) | To develop resistance against lepidopteran pests: multi-centric field trials in over 40 locations completed and further contained field trials in progress |
| M/s Rallis India Ltd, Bangalore | Chilli | Snowdrop (*Galanthus nivalis*) Lectin gene | Resistance against lepidopteran, coleopteran and homopteran pests; transformation experiments in progress |
| | Bell pepper | Snowdrop (*Galanthus nivalis*) Lectin gene | Resistance against lepidopteran, coleopteron and homopteran pests; transformation experiments in progress |
| | Tomato | Snowdrop (*Galanthus nivalis*) Lectin gene | Resistance against lepidopteran, coleopteran and homopteran pests; transformation experiments in progress |
| Indian Agricultural Research Institute, New Delhi | Brinjal, Tomato, Cauliflower | Bt gene<br>Bt gene<br>Bt gene | To impart lepidopteran pest resistance, transformation completed, green-house trials completed and one season field evaluation completed for brinjal and tomato |
| | Mustard/ rapeseed | Arabidopsis annexin gene | Transformation completed, green-house trial completed, ready for field-trials for moisture stress resistance |
| Jawaharlal Nehru University, New Delhi | Potato | Gene expressing for seed protein containing lysine obtained from seeds of *Amaranthus* plants (Ama-1 gene) | Transformation completed and transgenic potato under evaluation |

Table 2 — Summary of the biosafety information sought from GMO trials

| Particulars | Information sought |
|---|---|
| Rationale for the development | Economic, agronomic and other benefits, and rational of development |
| Details of the molecular biology of GMOs | • Description of the host plant<br>• Source and sequence of transgene<br>• Sequential block diagram of all trans-nucleic acid stretches inserted<br>• CI Cloning strategy<br>• Characteristics of expression vectors<br>• Characteristics of inserted genes with details of sequences<br>• Characteristics of promoters<br>• Genetic analysis including copy number of inserts, stability, level of expression of transgenes, biochemistry of expressed gene products, etc.<br>• Transformation/cloning methods |
| Laboratory and green-house trials | • Back-crossing methods<br>• Seed setting characteristics<br>• Germination rates<br>• Phenotypic characteristics<br>• Organism challenge tests wherever applicable<br>• Effects of chemical herbicides where ever applicable<br>• Toxicity and allergenicity implications if any during handling |
| Field trials | • Comparison of germination rates and phenotypic characteristics<br>• Study of gene flow<br>• Invasiveness studies<br>• Possibility of weed formation<br>• Possibility of transfer of transgenes to near relatives through out crossing<br>• Implications of out crossing<br>• Susceptibility to diseases and pests<br>• For human food/animal feed, composition and quality of transformed plants/fruits/seeds with controls<br>• Toxicity and allergenicity implications of plants/fruits/seeds and animals studies food/feed safety evaluation<br>• Handling procedures for allergenic substances<br>• Agronomic evaluation |

also been designed to study the potential for gene transfer and the consequence of transferring transgenic properties to weeds or other near relatives. The probability of pollen transfer and the natural mutation rates have been made conditions for computation in the experimental designs. The transgenic traits that have been looked at in such experiments in India include Bt-insect resistance, Bar resistance, Bar-barness as well as Bar-barster systems, Bar-Bt systems, antibiotic resistance, altered nutritional properties, and abiotic stress resistance properties.

As indicated earlier, data for submission by the applicants include mating systems in plants, comparison of germination rate, invasiveness, toxicity and allergenicity or alterations in the anti-nutritional properties of the plants due to the transgenes including the marker genes, etc. A detailed format for submitting information has been devised comprising nine chapters, and applicants are required to provide such information to the Government seeking exemption of the target transgenic plants for use in commercial agriculture under Rules 7,8,9,10 or 11 of the above Notification[2].

A few experimental designs have been evolved and approved by the RCGM for conducting trials in the open environment. The designs are for studying pollen dispersal, the comparison of cross- ability of non-transgenic plants with the transgenics and evaluation of their comparative competitiveness or invasiveness potential in unmanaged and managed land. The experimental results from two studies had shown that the pollen escape was a real phenomenon. The cross-ability studies conducted, e.g., on transgenic Indian mustard had shown that their existed pre- and post-fertilization barriers and the results corroborated the classical literature, confirming that escape of transgenes from same crops like the Indian mustard crop was not favoured in nature; however, viable F1 seeds could be produced by manual cross-pollination with related cultivated as well as wild species, which observation was consistent with similar studies made with *Brassica napus*[7]. It was observed, while studying the Bt cotton plants that their pollen also travelled to some distance with the help of insects. It can be stated from these that gene transfer shall be taking place in open environment when transgenic plants are cultivated. By appropriate management practices it might be possible to reduce the extent of pollen transfer into the open environment for all the crops, but it cannot be fully contained. Therefore the consequence of gene transfer is an issue which is real. The implications of this issue have not yet been satisfactorily resolved. A decision has to be taken by the Indian Government on this to decide to what extent transgene flow can be allowed and what are the consequential risks, taking also into consideration the agronomic benefits expected from the use of transgenic plants.

In addition to these experiments, major chunk of data are required to be generated on food safety in accordance with the latest Indian guidelines[5]. A schematic concept of the scientific need to generate safety information on transgenic food crops is shown in Figure 1. The information emphasizes quantitative production of transgenic proteins and their effects on as-is- where-is basis on experimental animals in the context of determining the toxicity allergenicity and anti-nutritional properties etc. The data generated in Indian experiments for Bt cotton, as well as for transgenic Indian mustard did not show any additional food safety risks.

The transgenic field experiments conducted in India have enabled the country to have hands-on experience on several genetically modified plants. Most important among them are transgenic Bt cotton, *Bar-Barnase* and *Bar-Barstar* mustard and Bt tomato. Data generated in India have demonstrated substantial agronomic benefits from transgenic plants over the corresponding non-transgenic controls. Table 3 provides an overview of the initial findings on the performance of the GMO plants.

In addition, experiments are also being convened for Bt cabbage, Bt cauliflower, Bt brinjal, Bt tobacco and

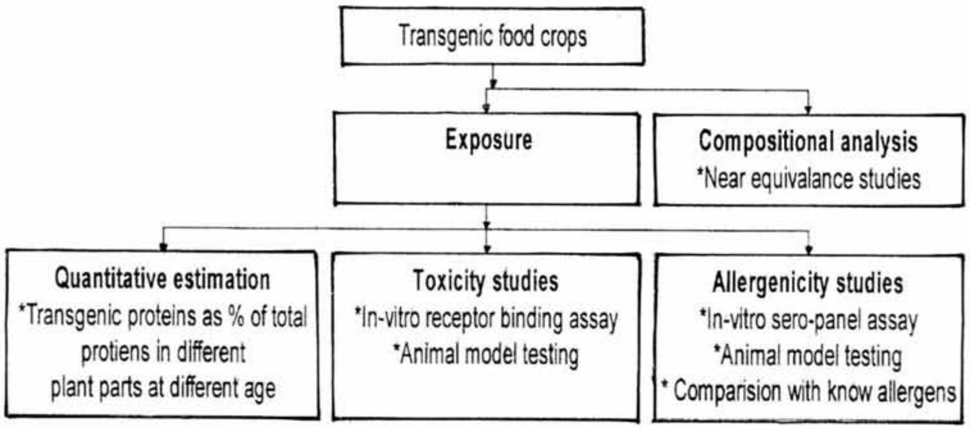


Figure 1 — Information needs for assessing safety of transgenic food crops

Table 3 — Percentage increase in productivity of transgenics over non-transgenics

| Name of the plant | Range of increase in productivities in per cent over controls |
|---|---|
| Bt cotton | 23 to 60 per cent: Average in 40 location study = 40 per cent |
| Bar-Barness-Barstar mustard | 5 to 43 per cent: Average in 15 location study = 16 per cent |
| Bt tomato | 300 per cent (one location study) |

Bt potato. Several other plants such as rice, pigeon pea, soybean, chili, bell pepper and corn have been transformed with improved traits and these are likely to be experimented upon in open environmental conditions soon.

## Conclusions

The Indian Government created the rules and procedures for dealing with GMOs in 1989. The first transgenic plant experiment in the field was started in 1995. Since then the country has acquired substantial experience in understanding the issues related to the handling of GMOs. Though no transgenic plant has yet been approved for commercial agriculture the rich experience gained from hands on experiments in field conditions has provided insights about the benefits as well as the risks from the use of such plants. On overall assessment, it appears that the benefits from the use of such plants are substantial and they may outweigh the risks which in many facets are presumptive and not real. With regard to actual risks, there are issues on the flow of transgenes into the open environment which are indeed real. The country has to decide how it would weigh the agronomic benefits vis-à-vis the issue of transgenic pollen escape into the environment.

## References

1 *Indian Environment (Protection) Act*, 1986.

2 Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Micro-organisms/Genetically engineered organisms or cells, issued by the Union Ministry of Environment and Forests, Government of India, vide Notification No. G.S.R. 1037 (E) dated 05 December 1989.

3 *Recombinant DNA Safety Guidelines*, 1990, issued by the Department of Biotechnology, Union Ministry of Science and Technology, Government of India in 1990.

4 *Revised Guidelines for Safety in Biotechnology*, issued by the Department of Biotechnology, Union Ministry of Science and Technology, Government of India in May 1994.

5 *Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants, and Plant Parts*, issued by the Department of Biotechnology, Union Ministry of Science and Technology, Government of India in August 1998 and partly amended on 24 September 1999.

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