Safe Movement of Transgenic Planting Material: Procedures and Challenges

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Transgenics have benefited the agriculture by introduction of superior traits for better agronomic performance, ensuring nutritional security and mitigating climate change. In India, to meet the demand of burgeoning population and to withstand the changing climate, GM crops would play an important role.

India ratified the Cartagena Protocol on Biosafety (CPB) way back on 23 January, 2003, since then the Ministry of Environment and Forests and Climate Change (MoEF&CC), Government of India is the nodal Ministry for effective implementation of the Protocol for safe transboundary movement of GMOs/living modified organisms (LMOs) (https://bch.cbd.int/).

National Regulatory Mechanism

In the country, regulatory framework is in place since 1989 under the provisions of the Environment Protection Act, 1986, by the MoEF&CC, Government of India. The Department of Consumers Affairs, Ministry of Consumer Affairs, Government of India, in an extraordinary gazette notification, made an amendment to enforce GM food labeling from January 2013 (Government of India Department of Consumer Affairs 2012) for selected food commodities so that consumers can opt for GM or non-GM products.

Regarding the safety of GM crops or products, these enter the fields or markets only after the thorough assessment for food and environment safety. An effective and stringent regulatory system for GM crops is already in place in the country, Randhawa & Chhabra 2009. This comprises of six competent authorities functioning in three-tier system: (i) Advisory Committee, (ii) Regulating and Approval Committees, and (iii) Post-Release Monitoring Committee.

I. Advisory Committee

i) Recombinant DNA Advisory Committee (RDAC): has an advisory role to review the recent developments in biotechnology at national and international levels.

II. Regulating and Approval Committees

ii) Institutional Biosafety Committee (IBSC): has both advisory and regulatory role and is constituted by respective organizations engaged in research on recombinant DNA technology. IBSC is responsible for compliance of guidelines at the Institute level.

iii) Review Committee on Genetic Manipulation (RCGM): has regulatory role and is responsible for Biosafety Research Level I field trials. RCGM regulates the research work at laboratory level through IBSC and at small-scale field trials of GM crops and directs generation of data on biosafety as per the guidelines or on case-by-case basis.
Monitoring and evaluation committee (MEC) is also in place to oversee the implementation of the project. To ensure safe crop yield, members of the committee conduct surprise field visits to check whether the correct procedure is being followed.

iv) Genetic Engineering Appraisal Committee (GEAC): is the regulatory authority for BRL II field trials. GEAC is the apex committee for authorizing release of GMOs and products thereof into the environment.

III. Post-Release Monitoring Committees

v) State Biotechnology Coordination Committee (SBCC): is constituted in each State where research and applications of GMOs are contemplated.

vi) District Level Committee (DLC): is constituted at district level to monitor the safety regulations pertaining to GMOs research and their applications.

Indian Council of Agricultural Research (ICAR)-National Bureau of Plant Genetic Resources (NBPGR) is the nodal agency for issuing Import Permit and for undertaking the quarantine processing of the imported transgenic planting material. This authorization has been vested upon ICAR-NBPGR vide Govt. of India Notification No. GSR 1067(E) dated 05.12.1989 and subsequently under the Plant Quarantine (Regulation of Import into India) Order 2003 of the Destructive Insects and Pests (DIP) Act, 1914. The Import Permit is issued by ICAR-NBPGR after the technical clearance for import is accorded by the Review Committee on Genetic Manipulation (RCGM) of Department of Biotechnology (DBT), Govt. of India.

As per the allocation of business, the research on transgenics comes under the purview of DBT and quarantine of imported germplasm/transgenic planting material for research comes under the mandate of the institute. Quarantine processing of imported transgenics is undertaken in the Containment Level–4 (CL-4) Facility. The facility has been built in a way that no viable biological material/ pollen/ pathogen can enter or leave. A fully functional Molecular Biology Laboratory was also established for testing of transgenes.

ICAR-NBPGR ensures prevention of entry of exotic pests (fungi, bacteria, viruses, insects, nematodes, weeds etc.) in imported germplasm/imported transgenics through stringent quarantine processing. Molecular testing for absence of terminator technology and declared transgenes is also undertaken in the material.

Since 1997, transgenic planting material is being imported through ICAR-NBPGR, along with quarantine processing of imported GMOs, molecular testing for specific transgenic elements as claimed by the importer/exporter is also carried out employing polymerase chain reaction (PCR) and real-time PCR based markers. Efficient detection strategies based on GMO matrix as a decision support system, loop-mediated isothermal amplification and multi-target real-time PCR-based systems have been developed. Till date, 227 consignments of fifteen GM crops namely, *Arabidopsis*, *Brassica* spp, including *B. oleracea var. capitata* and *var. botrytis, B. napus, B. juncea, Cicer arietinum* (chickpea), *Eucalyptus camaldulensis* (eucalyptus), *Glycine max* (soybean), *Gossypium* spp. (cotton), *Lycopersicon esculentum* (tomato), *Manihot esculenta* (cassava), *Nicotiana tabaccum* (tobacco), *Oryza sativa* (rice), *Solanum tuberosum* (potato), *Triticum aestivum* (wheat) and *Zea mays* (maize), have been imported from 19 countries by public and private sector.

At ICAR-NBPGR risk analysis for major pests (insects, mites, nematodes, bacteria, fungi, viruses and weeds) which may accompany the seeds of various crops from respective source countries, but not yet reported from India, it is being conducted on regular basis in a systematic manner through literature survey.

Quarantine processing: Seeds of transgenic material are being subjected to various tests in the containment for detection of insects, mites, nematodes, bacteria, fungi and viruses.

- Samples are examined visually under higher magnification for the associated pests and pathogens and subjected to blotter test after incubation at 20±1°C under fluorescent tubes in alternating cycles of 12 hours light/ darkness for 7 days to detect fungal pathogens.
- Chickpea, cotton and maize known to carry hidden infestation of insects are screened through soft X-ray radiography using a real-time X-ray machine for detection of insect groups that do not exhibit external symptoms of damage.
- Rice samples are subjected to soaking & washing tests to detect seed-borne nematodes.
- The material is also grown in the containment for detection of pests. Post-entry quarantine inspections of the imported transgenics is also undertaken at the indenter's site by the scientists of Plant Quaratine Division and the collected material is tested for exotic pests.
- Leaf samples showing viral symptoms are tested using transmission electron microscope, enzyme-linked immunosorbent assay (ELISA) and Reverse Transcription- Polymerase Chain Reaction (RT-PCR).

Some of Interception of pests: Important pests of quarantine significance intercepted were

- Fungi: *Alternaria brassicae, A. brassicicola, Phoma lingam* and *Xanthomonas campestris* pv. *campestris* in *Brassica juncea* from Australia; *Fusarium verticillioides, Pestalotiopsis theae* and *Verticillium albo-atrum* in cotton from USA; *F. verticillioides* in maize from the Philippines, South Africa, USA; *Alternaria padwickii* (China), *Drechslera oryzae (*Singapore), *Phoma glumarum* (Singapore), *V. cinnabarinum* (Vietnam) in rice; *Peronospora manshurica* (a fungus yet not known to occur in India) in soybean from USA and *Alternaria longipes* in tobacco from Canada.
- Insects: *Cryptolestes ferrugenius, Rhizopertha dominica* and *Sitophilus oryzae* in rice from Singapore and China.
- Nematode: *Aphelenchoides besseyii* in rice from the Philippines.
- Viruses (intercepted in PEQI) *Barley stripe mosaic virus, Maize dwarf mosaic virus* (MDMV) and *Wheat streak mosaic virus* in *Triticum aestivum* from USA; *Maize chlorotic mottle virus* in maize from Puerto Rico, MDMV and *Wheat streak mosaic virus* in maize from the Philippines were intercepted. Of these, *Maize chlorotic mottle virus* and *Wheat streak mosaic virus* are not yet reported from India and infected plants were uprooted and incinerated.

Salvaging of infected/infested material is undertaken using various salvaging techniques/treatments *viz*., mechanical cleaning, fumigation and X-ray radiography against insect pests, hot water treatment (HWT) and seed treatment against various seed-borne pathogens and nematodes. Rice samples are given mandatory HWT at 52^0C for 30 min. against seed-borne pathogens and nematode and tomato samples are given prophylactic treatment with 10% trisodium orthophosphate for externally seed-borne Tobamoviruses. http://www.nbpgr.ernet.in/Divisions_and_Units/Plant_Quarantine.aspx.

Only pest/ disease-free imported transgenics are released to the indentors. Interception of pests of quarantine significance resulted in protecting the crops against the ravages of these exotic pests which could have otherwise caused significant losses.

Decision support system for detection of imported transgenics

After receiving an imported sample, detection assays targeting commonly employed promoters, terminators and transgenes are performed to confirm its GM status, as claimed by the importer/exporter. Cost-effective strategies based on GMO matrix, polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP) and multi-target real-time PCR system, which facilitate testing of imported GMOs have been developed. To confirm the GM status of a sample irrespective of specific crop and GM trait, a hexaplex PCR-based screening assay targeting marker genes (*aadA, bar, hpt, nptII, pat, uidA*) commonly employed in imported GM events, was developed (Randhawa et al., 2009).

With the increase in number and diversification of genetic elements in GMOs, testing directly for presence/absence of each target is very challenging. "Matrix-based approach" is an efficient strategy to screen authorized GMOs and to detect accidental occurrence or adventitious presence of unauthorized GMOs (Holst-Jensen et al., 2012, Waiblinger et al., 2010). GMO matrix is used by selecting targets common to multiple GMOs, which would cut down cost of GM testing by eliminating the need of specific assays for every event. GMO matrix of 141 GM events of 21 crops with 106 genetic elements was developed as decision support system to check for authorized GM events in the country. Out of 141 GM events, more than 80 (55%) were imported (Randhawa et al., 2014a). The scope of this matrix would be further enhanced by adding information of upcoming new GM events.

TaqMan® real-time PCR based multitarget system simultaneously detecting 47 targets was developed (Randhawa et al., 2014b). Besides detection of commercialized GM events, the system allows detection of GM events (imported as single or stacked event) of maize (Bt11, Bt176,

MON810, MON89034, TC1507), cotton (MON531, MON15985, GFM-cry1A, MON1445, MON88913, Widestrike), rice (Liberty Link), soybean (GTS40-3-2) and wheat (MON71800) using event-/construct-specific assays. Imported events of other GM crops could be efficiently screened using assays, targeting commonly employed promoters, terminators and transgenes, included in the system.

LAMP assays were employed to detect commonly incorporated transgenic elements, *CaMV* 35S and *FMV* promoters, *aadA*, *nptII* and *uidA* marker genes, and *cry1Ac*, *cry2Ab2* and *cp4-epsps* genes to check the GM status of imported consignments (Randhawa et al., 2013; Singh et al., 2015).

The existing regulatory system for approval of GM crops is based on RCGM and GEAC, which have provided operational guidelines as per the best international norms such as guidelines by the Organisation for Economic Co-operation and Development (OECD).

For field trials or commercial adoption of GM crops, precautionary approach is undertaken, which does not allow such trials/ cultivation in the diversity-rich areas.

In view of the recent developments in the field of biotechnology, biosafety & biosecurity, and experience gained while implementing the biosafety frameworks within the country, a new guideline on 'Regulations and guidelines on biosafety of recombinant DNA research and biocontainment, 2017' has been prepared by Review Committee on Genetic Manipulation (RCGM), Department of Biotechnology, New Delhi. The guidelines prepared after due incorporation of views from researchers, experts, academicians, concerned Ministries/departments and other stakeholders, specifies practices for handling hazardous biological material, recombinant nucleic acid molecules and cells, organisms and viruses containing such molecules, in order to ensure an optimal protection of public health and of the environment. It provides clarity on competent authorities, biosafety requirements, recommendations for laboratory facilities such as facility design, biosafety equipment, personal protective equipment, risk assessment & management strategies, good laboratory practices & techniques, provisions for transboundary exchange of regulated materials, waste management etc

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