Transboundary Movement of Living Modified Organisms: Strengthening Capacities of Enforcement Agencies

Editors

V Celia Chalam
S C Dubey
C Murali Krishna
Shashi Bhalla
Kuldeep Singh

ICAR- National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi 110012

and

Ministry of Environment, Forest and Climate Change
Government of India, New Delhi 110003

Transboundary Movement of Living Modified Organisms: Strengthening Capacities of Enforcement Agencies

Editors

V Celia Chalam
S C Dubey
C Murali Krishna
Shashi Bhalla
Kuldeep Singh

ICAR- National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi 110012

and

Ministry of Environment, Forest and Climate Change
Government of India, New Delhi 110003

Citation: V Celia Chalam, S C Dubey, C Murali Krishna, Shashi Bhalla and Kuldeep Singh (eds.) (2018) Transboundary Movement of Living Modified Organisms: Strengthening Capacities of Enforcement Agencies. ICAR-National Bureau of Plant Genetic Resources and Ministry of Environment, Forest and Climate Change, New Delhi, India. vi+159 p.

ISBN 978-81-937111-2-5

Published in 2018

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
and
Ministry of Environment, Forest and Climate Change, New Delhi 110003, India

Copies can be obtained from:
The Director
ICAR-National Bureau of Plant Genetic Resources
New Delhi110012, India
Email:director.nbpgr@icar.gov.in
Website: http//:www.nbpgr.ernet.in

Printed by: Alpha Printographics (India); Mobile: 9999039940, 981 1199620

CONTENTS

S. No.	Title	Page No.
Foreword		v
Acknowledgements		vi
1	Introduction: Living Modified Organisms *V Celia Chalam, Shashi Bhalla and Kuldeep Singh*	1-11
2	Status of Genetically Modified Crops: Globally Approved Events *Shashi Bhalla, V Celia Chalam and Ruchi Sharma*	12-26
3	Cartagena Protocol on Biosafety: An Overview *Chimata Murali Krishna*	27-40
4	Indian Biosafety Regulatory Framework for Living Modified Organisms *Chimata Murali Krishna*	41-52
5	Role of Customs Officials in the Implementation of Cartagena Protocol on Biosafety *Chimata Murali Krishna*	53-61
6	National Plant Quarantine System *SC Dubey, V Celia Chalam and Shashi Bhalla*	62-76
7	Documentations Requirement for Transboundary Movement of Living Modified Organisms *Shashi Bhalla, V Celia Chalam, Ruchi Sharma and SC Dubey*	77-97
8	Use of Biosafety Clearing-House: An Information Portal on Living Modified Organisms *Vibha Ahuja*	98-111
9	Sampling Strategies: Bulk Material of Living Modified Organisms *V Celia Chalam, Shashi Bhalla, Chanda Priyadarshini and Ruchi Sharma*	112-125

10 Detection of Living Modified Organisms: Immunodiagnostics 126-139
V Celia Chalam, Shashi Bhalla, SC Dubey, Chanda Priyadarshini, Aradhika Tripathi, Ruchi Sharma and AK Maurya

11 Detection of Living Modified Organisms: DNA-based Techniques 140-147
Gurinderjit Randhawa and Monika Singh

Glossary 148-154

Acronyms 155-158

Contributors 159

Dr Sujata Arora
Adviser/ Scientist 'G'

सत्यमेव जयते

भारत सरकार
पर्यावरण, वन एवं जलवायु परिवर्तन मंत्रालय
GOVERNMENT OF INDIA
MINISTRY OF ENVIRONMENT, FOREST AND CLIMATE CHANGE
इंदिरा पर्यावरण भवन, जोर बाग रोड,
नई दिल्ली-110 003
INDIRA PARYAVARAN BHAWAN, JOR BAGH ROAD,
NEW DELHI-110 003
Website : moef.nic.in

FOREWORD

India is a Party to the Cartagena Protocol on Biosafety (CPB), an international agreement which aims to ensure safe handling, transport, packaging and identification of living modified organisms (LMOs) resulting from modern biotechnology and their transboundary movement that may have adverse effects on biological diversity, taking also into account risks to human health. Implementation of CPB in India for safe transboundary movement of LMOs requires strengthening the capacities of enforcement agencies including plant quarantine and customs official, which are the first line of defence.

Towards this, the Ministry of Environment, Forest and Climate Change (MoEFCC) as the nodal Ministry for implementation of CPB, implemented a United Nations Environment Programme-Global Environment Facility (UNEP-GEF) Phase-II Capacity Building Project on Biosafety for the thematic area "Handling. Packaging, Transport and Identification of LMOs (Article 18) of CPB". Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR-NBPGR), which is the nodal organisation for management of plant genetic resources (PGRs) including transgenics and is vested with the authority to issue Import Permit and Phytosanitary Certificate, and undertake quara ntine of PGRs including transgenics was engaged as one of the partnering agencies in implementation of this project.

ICAR-NBPGR and MoEFCC in association with the National Academy of Customs, Indirect Taxes & Narcotics (NACIN) and Customs organized 14 Training Workshops during 2015-2018 on Strengthening Capacities of Enforcement Agencies (Plant Quarantine and Customs) for Transboundary Movement of LMOs for plant quarantine and customs officials. The Training Workshops covered a wide spectrum of topics including National and International Framework on Biosafety of LMOs, Role of Customs Officials in the implementation of CPB, National Plant Quarantine System, Documentation Requirements for Transboundary Movement of LMOs, Sampling Strategy for LMOs, Use of Biosafety Clearing-House, and Detection of LMOS (Immonodiagnostics and PCR-based).

I commend the efforts of all concerned in bringing out this book based on the lectures delivered on different topics in the Training Workshops. I hope this publication would serve as a useful resource and reference material for quarantine and customs officials and for the researchers specialized in the area of plant quarantine and biosafety of LMOs.

प्रकृति: रक्षति रक्षिता
Nature Protects if She is Protected

Sujata Arora

Dr Sujata Arora

ACKNOWLEDGEMENTS

We express our sincere gratitude to Dr. K.C. Bansal, Former Director and Former Project Coordinator, ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi for his keen interest and encouragement in getting the Project entitled "Strengthening Capacities of Enforcement Agencies (Plant Quarantine and Customs Officials) for Transboundary Movement of LMOs", a UNEP-GEF Phase –II Capacity Building Project on Biosafety, at ICAR-NBPGR.

We are highly indebted to Sh. Hem Pandey, Additional Secretary and Dr. Ranjini Warrier, Adviser, MoEF&CC for their constant support and guidance for organizing Training Workshops. Our special thanks are due to Dr. Amita Prasad, Additional Secretary, MoEF&CC and Sh. Gyanesh Bharti, Joint Secretary, MoEF&CC for extending the project and giving us the opportunity to organize the Training Workshops at different ports. We are grateful for the support of Dr. Madhumita Biswas, Director, MoEF&CC and Dr. Sujata Arora, Adviser, MoEF&CC for organizing Training Workshops and completing this project.

We thank Dr. Alex Owusu Biney, UNEP, Portfolio Manager and GEF for their constant guidance and financial support.

The editors are very grateful to Dr. Vibha Ahuja, Chief General Manager, Biotech Consortium India Ltd. (BCIL), staff of BCIL, the Project Coordinating Unit for all the support since the inception to completion of the project.The project team sincerely thank the National Academy of Customs, Excise and Narcotics, now National Academy of Customs, Indirect Taxes & Narcotics (NACIN) and Customs for facilitating the organisation of Training Workshops in different parts of the country which would not have been possible without their support. The sincere efforts of Ms. Chanda Priyadarshini, Ms. Helena Shepherou, Dr. Aradhika Tripathi, Dr. Ruchi Sharma and Ms. Seema Goswami for organizing Training Workshops and bringing out this publication are duly acknowledged. The editors are extremely grateful to the contributing authors for delivering the lectures and giving the manuscript.

Editors

Introduction: Living Modified Organisms

1

V Celia Chalam, Shashi Bhalla and Kuldeep Singh
ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
Email: *celia.chalam@icar.gov.in; mailcelia@gmail.com*

1. Introduction

The world food production will have to be doubled by 2050 to meet rising demand of increased population and income growth according to the World Bank. To meet the target, a comprehensive effort needs to be made in all directions for combating factors that limit food production and supply. Many observers have suggested that biotechnology has the potential to increase world food output and reduce food insecurity by improving crop yields and reducing crop losses. By adding genes to conventional crops to help them resist insect pests, diseases, or drought, crops can be produced that use less of an expensive input or crops that produce higher yields. Any one, or several, of these improvements can be tailored to make individual crops more likely to thrive in a particular country's growing conditions, and can potentially allow a wider variety of innovations. Genetically modified (GM) crops could contribute to a more sustainable agricultural system.

The application of modern biotechnology has considerable potential for enabling improvements in a variety of fields, from medicine to agriculture and from management of pollution to industrial production. However, on the other side there are issues including ethical considerations and the possible risks to human health and the environment. The perceived lack of experience with the technology compounds these potential concerns, including insufficient information on the long-term effects of these organisms, if released into the environment and the possible serious effects on biodiversity.

Living Modified Organism (LMO) is any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (recombinant DNA technology) and living organism means biological entity capable of transferring or replicating genetic material. In general the term LMO is considered to be functionally the same as genetically modified organism (GMO). Many countries use the terms GMO, and 'transgenic organism' in domestic legislation to describe LMOs. As given by the Secretariat of the Convention on Biological Diversity (CBD), these terms can therefore be used interchangeably. However, the Cartagena Protocol on Biosafety, an International Protocol

specifically uses the term LMO. Most of the LMOs that have been developed to date are agricultural crops.

Agricultural biotechnology became commercially available in 1996, and has since been used safely and successfully by thousands of farmers in the US and 24 other countries including India (ISAAA, 2017). This major scientific advancement has made plants resistant to insect pests and diseases in ways that have never before been achieved, thereby also increasing crop yields. Thus, technology contributes to increase world food output and reduce food insecurity. However, there is a need to address areas of concern with biotechnology. Biosafety of genetically engineered organisms is a major concern that must be addressed cautiously with appropriate scientific analysis of the types of risks these organisms may pose. The biosafety concerns related to transgenic crops are environmental, health, socio-economic and ethical. Social and ethical concerns are guided partly by religious beliefs, customs and cultures, and partly by the conviction. The environmental issues are concerning gene flow from crop to crop, gene flow from crop to weed, impact on biodiversity, possible weediness, persistence or invasiveness of crops, horizontal gene transfer, evolution of super pests or diseases, non-target effects and health related issues such as allergenicity and toxicity of GM crops.

Current applications of GM technology in agriculture suggest that, when properly managed, GM crops can deliver their potential to reduce the environmental impact of farming. A drought-tolerant corn variety has been developed which can provide about 10% increase in yield in less water conditions. Such innovations have potential to contribute to poverty reductions and food security. Thus, GM crops could contribute to a more sustainable agricultural system.

2. Global Status of GM Crops

As a result of consistent and substantial benefits during the 22 years of commercialization from 1996 to 2017, farmers have continued to plant more biotech/GM crops every single year. The global area of GM crops in 2017 increased to189.8 million hectares. In 2017, the number of countries planting biotech crops are 24 and comprised 19 developing countries and five industrial countries; they were, in order of hectarage, USA, Brazil, Argentina, Canada, India, Paraguay, Pakistan, China, South Africa, Bolivia, Uruguay, Australia, the Philippines, Myanmar, Sudan, Spain, Mexico, Colombia, Vietnam, Honduras, Chile, Portugal, Bangladesh and Costa Rica. Developing countries grew 53% (100.6 million hectares) of the global biotech crop area compared to 47% for industrial countries. An additional 43 countries (17 + 26 EU countries) formally imported biotech crops for food, feed and processing. Thus, a total of 67 countries have adopted biotech crops. Biotech crops have expanded beyond the major four (corn, soybeans, cotton and canola) to give more choices for many of the world's consumers

and food producers. These biotech crops include alfalfa, sugar beet, papaya, squash, eggplant, potato and apple, all of which are already in the market. Two generations of Innate® potatoes with non-bruising, non-browning, reduced acrylamide and late blight resistant traits were planted in the USA and Canada, and non-browning apples in the USA. Bt eggplant adoption in Bangladesh increased to 2,400 hectares on its fourth year of commercialization, 25 hectares of biotech pink pineapple in Costa Rica, increased ear biomass and high amylose content maize, and soybean with modified oil content. An insect resistant sugarcane was approved by Brazil for commercialization in 2018. Additionally, biotech crops research conducted by public sector institutions include rice, banana, potato, wheat, chickpea, pigeonpea, mustard, cassava, cowpea, and sweet potato with various economically important and nutritional quality traits beneficial to food producers and consumers in developing countries (ISAAA, 2017).

This major scientific advancement has made plants resistant to insect pests and diseases in ways that have never been achieved before, thereby also increasing crop yields. But due to unwarranted political stigmatization and unfounded scientific criticism, public funding and regulatory approvals for biotech crops have been hindered in many countries. They are often developing countries, where these technological advancements are most urgently needed.

3. International Regulation of LMOs

LMOs including GM crops are regulated which are governed under the Cartagena Protocol on Biosafety (CPB). Transboundary movement of LMOs/ GM crops is also regulated by the Protocol. The Protocol was adopted by the CBD inSeptember 2000 and came into force in September 2003. The objective of the Protocol is to protect biological diversity from the potential risks posed by safe transfer, handling and use of LMOs resulting from modern biotechnology. According to the CPB, LMO is any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (recombinant DNA technology) and living organism means biological entity capable of transferring or replicating genetic material.

Risks to human health are also considered. The Protocol is applicable to all LMOs, except pharmaceuticals for humans that are addressed by other international agreements or organisations.

The Protocol sets out an Advance Informed Agreement (AIA) procedure for LMOs intended for intentional introduction into the environment that may have adverse effects on the conservation and sustainable use of biodiversity. The procedure requires, prior to the first intentional introduction into the environment of an importing party:

- Notification of the party of export containing certain information
- Acknowledgement of its receipt
- The written consent of the party of import

Four categories of LMOs are exempted from the AIA: LMOs in transit, LMOs for contained use, LMOs identified in a decision of the Conference of Parties/meeting of Parties as not likely to have adverse effects on biodiversity conservation and sustainable use, and LMOs intended for direct use as food, feed or for processing.

For LMOs that may be subject to transboundary movement for direct use as food, feed, or for processing, Article 11 provides that a party that makes a final decision for domestic use, including placing on the market, must notify the Biosafety Clearing-House (BCH) established under the Protocol. The notification is to contain minimum information required under Annex II of the Protocol. A contracting party may take an import decision under its domestic regulatory framework, provided this is consistent with the Protocol. A developing country contracting party, or a party with a transition economy that lacks a domestic regulatory framework, can declare through the BCH that its decision on the first import of an LMO for direct use as food, feed or for processing will be pursuant to a risk assessment. In both cases lack of scientific certainty because of insufficient relevant scientific information and knowledge regarding the extent of potential adverse effects shall not prevent the contracting party of import from taking a decision, as appropriate, in order to avoid or minimize potential adverse effects.

Risk assessment and risk management are requirements for both AIA and Article 11 cases. In principle, risk assessment is to be carried out by competent national decision-making authorities. The exporter may be required to undertake the assessment. The Protocol specifies general risk management measures and criteria. Any measure based on risk assessment should be proportionate to the risks identified. Measures to minimize the likelihood of unintentional transboundary movement of LMOs are to be taken. Affected or potentially affected states are to be notified when an occurrence may lead to an unintentional transboundary movement.

The Protocol also contains provisions on **LMO Handling, Packaging and Transportation** (Article 18). In particular, each contracting party is to take measures to require documentation that:

(a) For LMOs intended for direct use as food or feed, or for processing, clearly identifies that they "may contain" LMOs and are "not intended for intentional introduction into the environment", and a contact point for further information;

(b) For LMOs destined for contained use, clearly identifies them as LMOs and specifies any requirement for safe handling, storage, transport and use, and a contact point and consignee;

(c) For LMOs intended for intentional introduction into the environment of the party of import, clearly identifies them as LMOs and specifies the identity and traits/ characteristics, any requirements for safe handling, storage, transport and use, and a contact point, the name/ address of the importer/ exporter and a declaration that the movement conforms to the Protocol's requirements applicable to the exporter.

Information exchange is also envisaged in the Protocol through the establishment of BCH. The BCH is intended to facilitate the exchange of information on, and experience with, LMOs and to assist parties in implementation of the Protocol. Pursuant to Article 20, paragraph 2, it shall also provide access to other international biosafety information exchange systems. Information that parties are required to provide to the BCH includes existing laws, regulations and guidelines for implementation of the Protocol; information required for the AIA; any bilateral, regional and multilateral agreements within the context of the Protocol; summaries of risk assessment and final decisions.

Public participation is specifically addressed in Article 23. Contracting parties shall:

(a) Promote and facilitate public awareness, education and participation concerning safe transfer, handling and use of LMOs;

(b) Endeavour to ensure public awareness and education encompasses access to information on LMOs identified by the Protocol that may be imported;

(c) Consult the public in the decision-making process regarding LMOs and shall make decisions available to the public in accordance with national laws and regulations. Confidential information is to be respected in those activities.

Socio-economic considerations are allowed in decision-making. Contracting parties may account for socio-economic considerations arising from the impact of LMOs on biodiversity conservation and sustainable use, especially with regard to the value of biodiversity to indigenous and local communities. The parties are encouraged to cooperate on research and information exchange on any socio-economic impacts of LMOs (http://www.biodiv.org/ biosafety).

4. National Status of GM Crops

In India, cotton is the only GM crop deregulated for commercial cultivation. India achieved

a great stride in cotton production with a quarter of market share in global cotton production in 2017. Biotech cotton area increased by 6% from 10.8 million hectares in 2016 to 11.4 million hectares in 2017, equivalent to 93% of total cotton area of 12.24 million hectares. Insect resistant (Bt) technology in cotton hybrids delivered broad based benefits by saving losses caused by American bollworm and boosting cotton yield to 500 kg lint per hectare (ISAAA, 2017). More than 1,167 Bt cotton hybrids are approved for cultivation in India from 2002 onwards (Choudhary and Gaur, 2015). The reason for the spectacular growth in Bt cotton is that it has consistently delivered unprecedented benefits to farmers and to the nation. The field trials of GM crops containing new genes/events are given in Table 1.

Table 1. Field trials of GM crops containing new genes/ events during 2013

S. No.	Crop	Company name	Trial	Trait	Gene/ event
1.	RRF cotton	Maharashtra Hybrid Seeds Company Ltd.	BRL-1 2nd year	Herbicide tolerance	*cp4epsps/* MON 88913
2.	Herbicide tolerant Glytol cotton	Bayer Bioscience Pvt. Ltd.	BRL-1 2nd season	Herbicide tolerance	*2mepsps* (Event GHB 614)
3.	Twilink cotton	Bayer Bioscience Pvt. Ltd.	BRL-1	Insect resistance	Stacked events viz., GHB119 *(cry2Ae/ PAT)* & T304-40 *(cry1Ab/PAT)* containing *cry1Ab, cry2Ac* and *bar*
4.	Corn	Syngenta Biosciences Pvt. Ltd. Syngenta Biosciences Pvt. Ltd.	BRL-1 BRL-1 2nd year	Insect resistance and herbicide tolerance Insect resistance and herbicide tolerance	Events Bt11, GA21 and stack of Bt11xGA21 Bt11, GA21 and stack of Bt11xGA21

		Monsanto India Ltd.	BRL-1 2nd year	Insect resistance	*cry2Ab2* and *cry1A. 105 (Event MON 89034)*
5.	Herbicide tolerant corn	Monsanto India Ltd.	BRL-1 2nd year	Herbicide tolerance	*cp4 epsps* (event NK603)

BRL: Biosafety Research Level
Source: http://igmoris.nic.in/field_trials.asp

5. National Regulatory Mechanism for GM Crops

The Government of India has a comprehensive framework for ensuring safety while dealing with transboundary movement of LMOs/GMOs. The relevant domestic regulations and guidelines involving the import and export of LMOs include the following:

5.1. Regulations/ Guidelines

- Environment (Protection) Act,1986
- Rules for the Manufacture, Use/Import/Export and Storage of Hazardous Micro Organisms/ Genetically Engineered (GE) Organisms or Cells, 1989
- Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant Parts (1998)
- Guidelines for Institutional Biosafety Committees (IBSCs), 2011
- Plant Quarantine (Regulation of Import into India) Order, 2003 (PQ Order 2003)
- Notification relating to Inclusion of GM Policy in the Foreign Trade Policy (2006-09)
- Biological Diversity Act, 2002
- Guidelines for Environmental Risk Assessment of Genetically Engineered Plants, 2016
- Regulations and Guidelines for Recombinant DNA Research and Biocontainment, 2017

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 meant for research purposes. This authorization has been vested upon ICAR-NBPGR vide Govt. of India Notification No. GSR 1067(E) dated 05.12.1989 and PQ Order 2003. ICAR-NBPGR issues Import Permit only after Review Committee on Genetic

Manipulation of the Department of Biotechnology has accorded the import clearance. ICAR-NBPGR also issues Phytosanitary Certificate for transgenic germplasm meant for export.

6. Preparedness by ICAR-NBPGR

- Developed system for testing of transgenic planting material in quarantine (transboundary movement)
- Developed system for testing for transgene/ terminator gene technology
- Established Containment Facility (CL-4) to deal with transgenics
- Established Transgenic crops germplasm bank
- Capacity building since 2000 – Orientation Courses on Biosafety Considerations of Transgenics, Detection of GMOs and Training Workshops for Enforcement Agencies (Plant Quarantine and Customs Officials) on Transboundary Movement of LMOs
- Developed documentary film (DVD) on Safe Movement of Transgenics and Detection of GMOs

7. Recombinant Therapeutics Approved for Marketing in India

In India, there are recombinant therapeutics approved for marketing and the details are given in Table 2.

Table 2. Recombinant therapeutics commercially approved for marketing in India

S. No.	Molecules	Therapeutic indications
1.	Human insulin	Diabetes
2.	Erythropoietin	Treatment of anaemia
3.	Hepatitis B vaccine (recombinant surface antigen based)	Immunization against Hepatitis B
4.	Human growth hormone	Deficiency of growth hormone in children
5.	Interleukin 2	Renal cell carcinoma

6.	Granulocyte colony stimulating factor (GCSF)	Chemotherapy induced neutropenia
7.	Granulocyte macrophage colony stimulating factor (GMCSF)	Chemotherapy induced neutropenia
8.	Interferon 2alpha	Chronic myeloid leukemia
9.	Interferon 2beta	Chronic myeloid leukemia, Hepatitis B and Hepatitis C
10.	Interferons gamma	Chronic granulomatous disease and Severe malignant osteopetrosis
11.	Streptokinase	Acute myocardial infarction
12.	Tissue plaminogen activator	Acute myocardial infarction
13.	Blood factor VIII	Haemophilia type A
14.	Follicle stimulating hormone	Reproductive disorders
15.	Teriparatide (Forteo)	Osteoporosis
16.	Drotrecogin (Xigris) alpha	Severe sepsis
17.	Platelet derived growth factor (PDGF)	Bone marrow induction and osteoblasts proliferation
18.	Epidermal growth factor (EGF)	Mitogenesis and organ morphogenesis
19.	Eptacogalpha (r-F VIIa) r-coagulation factor	Haemorrhages, congenital or acquired hemophilia
20.	Bevacizumab	Treatment of various cancers, including colorectal, lung and kidney cancer
21.	Trastuzumab	Treatment of breast cancer
22.	Rituximab	Treatment of many lymphomas, leukemia, transplant rejection and some autoimmune disorders

23.	Darbopoetin alpha	Treatment of anaemia
24.	Human serum albumin	Treatment of liver disease with ascites
25.	Insulin glargin	Treatment of Type I Diabetes mellitus
26.	Insulin lispro	Treatment of Diabetes mellitus
27.	Insulin aspart	Treatment of Diabetes mellitus
28.	Met-hu-GCSF	Chemotherapy induced neutropenia
29.	Peg-r-metHu-GCSF	Chemotherapy induced neutropenia
30.	Human interferon alpha 2b	Treatment of chronic Hepatitis B
31.	Peg-Interferon alpha-2b	Treatment of chronic Hepatitis B
32.	Human INF beta-1a	Treatment of multiple sclerosis (MS)
33.	Peg Human GCSF	Chemotherapy induced neutropenia
34.	Human PDGF-BB-beta-TCP	Bone marrow induction and osteoblasts proliferation
35.	r-Hu-chorionic gonadotropin Hormone	Role in pregnancy
36.	Hemophilic factor IX	Treatment of hemophilia
37.	Cetuximab	Treatment of metastatic colorectal cancer and head and neck cancer
38.	Luteinising hormone	Treatment of reproductive disorders

8. Perspectives

With the advent of GM crops now the private sector is developing biotech crops that need less fertilizer, corn that more efficiently can be turned into ethanol and biotech canola that performs well even in drought conditions. The public sector in India is also investing a lot in developing GM crops and the pace of research outcome seems to be faster than the regulatory approvals. Besides, lot of work still remains to be done for the large scale public acceptance of the GM crops and food.

The world will be simply unable to address the growing demand for food unless farmers everywhere are able to produce more, with greater efficiency and GM crops are likely to play an important role in this direction. However, claims that only GM crops will end hunger through increased production perpetuate the myth that hunger is caused by scarcity of food. More than enough food is already produced to feed everyone in the world but people still go hungry. People don't have food in sufficient quantity or quality because they lack money to buy food or they are deprived of the means to produce it themselves. Hunger is inextricably linked with poverty. Poverty and hunger result from trade and economic policy decisions that lead to increasing inequalities in distribution of income and food.

Intellectual property rights and rights of access to and ownership of genetic resources are other key issues to be addressed. They raise difficult political problems, which are made more complex by the emergence of many private actors and which need to be resolved to maximize the potential benefits of biotechnology in agricultural research. The potential of GM crops may be further exploited which have an important role in food security and sustainable agriculture.

9. References

- Choudhary B and Gaur K (2015) *Biotech Cotton in India, 2002 to 2014*. ISAAA Series of Biotech Crop Profiles. ISAAA: Ithaca, NY.
- http://envfor.nic.in/legis/hsm/hsm3.html. Rules for the Manufacture, Use, Import, Export And Storage of Hazardous Micro Organisms, Genetically Engineered Organisms or Cells. 1989. Retrieved March 25, 2018.
- http://igmoris.nic.in/default.asp. IGMORIS. Retrieved May 27, 2016.
- http://www.biodiv.org/biosafety. The Cartagena Protocol on Biosafety. Retrieved March 25, 2018.
- ISAAA (2017) *Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years.* ISAAA Brief No. 53. ISAAA: Ithaca, NY. http://www.isaaa.org/ Retrieved June 28,2018.

Status of Genetically Modified Crops: Globally Approved Events

2

Shashi Bhalla, V Celia Chalam and Ruchi Sharma

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
Email: *sbhallamail@gmail.com*

1. Introduction

The world population is increasing at a very fast pace. Feeding 9.7 billion people in 2050 and ~11.0 billion in 2100 is a major challenge facing the mankind. Globally, 870 million people are chronically hungry and two billion are malnourished. The conventional crop technology may not feed over 9 billion in 2050. Therefore, conventional crop technology (well adapted germplasm) and biotechnology (genetically modified (GM) crops with desirable traits) may contribute to achieve sustainable intensification of crop productivity. The data of 1996-2017 has shown that GM/ biotech crops contributed to food securityand sustainability by increasing crop production.

Biotechnology is a revolutionary technology which can change the characteristics of living organisms by transferring the genetic information from one organism, across species boundaries, into another organism. Biotechnology identifies desirable traits more quickly and accurately than conventional plant breeding and allows gene transfers which are impossible with traditional breeding. This offers opportunities for development of GM crops in which the genes or genetic material of the organism has been modified in a way that does not occur naturally. The GM crops are the ones that have been transformed by the insertion of one or more transgenes. The GM crops are being developed with important traits viz., agronomic traits, resistance to insect pests, diseases, tolerance to herbicides, abiotic stresses, improvement of quality traits and manipulation of physiological attributes for greater productivity and sustainability. The first generation of GM crops targeted input traits of herbicide tolerance (HT), insect resistance (IR) and virus resistance where farmers and food producers were benefited and also provided food and nutrition for the 7.4 billion population globally. The second generation GM crops include stacks of these traits, as well as drought tolerance – one of the problems related to climate change. Adoption of IR/HT soybean (Intacta™) and corn rootworm stacks for maize have been phenomenal with an economic benefit of US$2.4

billion in 2013-2015 and US$12.6 billion in 2003 to 2015, respectively (Brookes and Barfoot, 2018). Output traits for improved quality and composition were the traits of the third generation GM crops which contributed towards consumer preference and nutrition.

Every cell that successfully incorporates the gene of interest represents a unique "event" and an event is the insertion of a particular transgene into a specific location on a chromosome. For example, two lines of the same plant species transformed with the same or different constructs constitute two events. Every plant line derived from a transgenic event is considered a GMO. Different events can have much different consequences. This depends on the number of times the gene construct was added to the cell's genome and may also have something to do with the placement of the new genes. Events can be introduced to other cultivars by breeding. This is why certain events (e.g. MON 810) are available in many different cultivars.

The UN Food and Agriculture Organization, International Food and Policy Research Institute, the G20 countries and other like-minded bodies, guided by 2030 Agenda for Sustainable Agriculture have committed to eradicate hunger and malnutrition in 15 years or less. More importantly, the US National Academies of Sciences, Engineering, and Medicine published a review of 900 researches on GM crops since 1996 and found that GM crops and conventionally-bred crops have no difference in terms of probable risks to human health and the environment. GM crops now have an unblemished record of safe use and consumption for over 20 years. Future generations can benefit more from wide choices of GM crops with improved traits for high yield and nutrition as well as safe for food use and environment (ISAAA, 2016). In 2017, ~17 million farmers in 24 countries (19 Developing and 5 Industrial) planted 189.8 million hectares of GM crops. Globally, 12 crops with approved events are under cultivation in different countries.

GM crops are being adopted globally because of the enormous benefits to the environment, health of humans and animals, and contributions to the improvement of socio economic conditions of farmers and the general public. GM crops contributed to food security, sustainability and climate change by: increasing crop productivity by 657.6 million tons valued at US$186.1 billion in 1996-2016; conserving biodiversity in 1996 to 2016 by saving 183 million hectares of land, providing a better environment by saving on 671 million kg a.i. of pesticides in 1996-2016, and by 48.5 million kg in 2016 alone from being released into the environment; by saving on pesticide use by 8.1% in 2016 alone; by reducing Environmental Impact Quotient (EIQ) by 18.4% in 1996-2016, and by 18.3% in 2016 alone; reducing CO_2 emissions in 2016 by 27.1 billion kg, equivalent to taking 16.7 million cars off the road for one year; and helping alleviate poverty through uplifting the economic situation of 16-17 million small farmers, and their families totaling >65 million people,

who are some of the poorest people in the world (Brookes and Barfoot, 2018, Forthcoming). By using these technologies, small poor farmers will be able to survive and contribute to the doubling of food production to meet the needs of a growing population which will reach over 11 billion in 2100 (ISAAA, 2016).

2. Global Status of Genetically Modified Crops

The year 2016 has marked the 21st anniversary (1996-2016) of the commercialization of biotech/ GM/ transgenic crops. The GM crops delivered substantial agronomic, environmental, economic, health and social benefits to farmers and increasingly society at large. The GM crops planting in 2017 resumed high adoption (17 million farmers) in 24 countries at 189.8 million hectares worldwide, showing an increase of 5.4 million hectares or 3% from 185.1 million hectares in 2016. The GM crops have expanded beyond the four major crops viz., corn, soybean, cotton and canola to include more diverse crops such as alfalfa, sugar beet, papaya, squash, eggplant, potato and apple. Potato is the fourth important staple crop in the world and eggplant is the number one vegetable consumed in Asia. Additionally, public sector institutions are taking up research on crops like rice, banana, potato, wheat, chickpea, pigeonpea, mustard, cassava, cowpea and sweet potato with various economically important and nutritional quality traits beneficial to food producers and consumers in developing countries. Two generations of Innate ® potatoes with no-bruising, non-browning, reduced acrylamide and late blight resistant traits were planted in USA and Canada, and non-browning apples in the USA. Bt eggplant adoption in Bangladesh increased to 2400 hectares on its fourth year of commercialization. IR sugarcane has been approved by Brazil for commercialization in 2018. Area and different crops grown by different countries are given in Table 1.

Table 1. Global area of GM crops in 2017: Country-wise (million hectares) **

Rank	Country	Area (million ha)	GM Crops
1	USA*	75.0	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash, potato, apples
2	Brazil*	50.2	Soybean, maize, cotton
3	Argentina*	23.6	Soybean, maize, cotton
4	Canada*	13.1	Canola, maize, soybean, sugar beet, alfalfa, potato

5	India*	11.4	Cotton
6	Paraguay*	3.0	Soybean, maize, cotton
7	Pakistan*	3.0	Cotton
8	China*	2.8	Cotton, papaya
9	South Africa*	2.7	Maize, soybean, cotton
10	Bolivia*	1.3	Soybean
11	Uruguay*	1.1	Soybean, maize
12	Australia*	0.9	Canola, cotton
13	Philippines*	0.6	Maize
14	Myanmar*	0.3	Cotton
15	Sudan*	0.2	Cotton
16	Spain*	0.1	Maize
17	Mexico*	0.1	Cotton
18	Colombia*	0.1	Cotton, maize
19	Vietnam	<0.1	Maize
20	Honduras	<0.1	Maize
21	Chile	<0.1	Maize, canola, soybean
22	Portugal	<0.1	Maize
23	Bangladesh	<0.1	Brinjal/eggplant
24	Costa Rica	<0.1	Cotton, pineapple
Total		**189.8**	

*18 biotech mega-countries growing 50,000 hectares, or more, of GM crops

**Rounded-off to the nearest hundred thousand

Source: ISAAA (2017)

The global area of GM crops has increased ~112 fold from 1.7 million hectares in 1996 to 189.8 million hectares in 2017 and this makes GM crops the fastest adopted crop technology in recent times. Of the top five countries growing 91% of GM crops, three are developing countries (Brazil, Argentina and India) and two are industrial (USA and Canada). USA leads biotech crop planting in 2017 at 75 million hectares, followed by Brazil (50.2 million hectares), Argentina (23.6 million hectares), Canada (13.1 million hectares) and India (11.4 million hectares) (Table 1) for a total of 173.3 million hectares representing 91.3% of the global area and benefiting more than 1.95 billion people in the five countries. An accumulated 2.3 billion hectares or 5.9 billion acres was achieved in 22 years (1996-2017) of GM crop commercialization. The average GM crop adoption rate in the top five GM-crops growing countries increased in 2017 and reached close to saturation with USA at 94.55% average for soybean, maize and canola, Brazil-94%, Argentina ~100%, Canada-95% and India -93%.

The four major GM crops viz., soybean, maize, cotton and canola, in decreasing area, were the most adopted GM crops by 24 countries. The area planted to GM soybean was the highest at 94.1 million hectares, which is 50% of the global hectarage for all GM crops. This is followed by maize (59.7 million hectares) and canola (10.2 million hectares). Based on the global crop hectarage for individual crops, 77% of soybean, 80% of cotton, 32% of maize and 30% of canola were GM in 2017.

The commercialized GM crops are with the genetically altered traits viz., alfalfa, soybean and sugar beet with herbicidal resistance; cotton and maize with IR and HT; brinjal/eggplant and potato for IR; papaya and squash for virus resistance and canola/ rapeseed with altered oil composition, high lauric acid content and resistant to glufosinate for male sterility properties. The area planted with GM crops with stacked traits increased by 3% and covered 41% of the global area which reduced the insecticide use. Herbicide tolerance in soybean, canola, maize, alfalfa and cotton, has consistently been the dominant trait which in 2017 covered 47% of the global area-an increase of 2% compared to 2016.

Globally, USA continued to be the leader in commercial cultivation of GM crops since 1996. In 2017, GM crops planted in USA were the highest globally at 75.04 million hectares comprising 34.05 million hectares soybean, 33.84 million hectares maize, 4.58 million hectares cotton, 1.22 million hectares alfalfa, 876,000 hectares canola, 458,000 hectares sugar beet, 3000 hectares potato and some 1000 hectares each of GM apple, squash and papaya. Generally, the area planted to biotech crops increased in the USA except for maize and sugar beet. The lesser drought incidence and lesser storms that bypassed the crop growing areas across the country as well as the favourable and profitable prices for soybean, cotton and canola were incentives for the farmers to increase the area of these three GM crops and has

reached the near adoption rate of 94.5% from the three major crops: maize, soybean and cotton. Thus, further expansion in GM crop area depends on the adoption of other GM crops: canola, alfalfa, sugar beet, potato and apple. The current revamp on GM regulations of the regulatory agencies reflect country's leadership in acceptance and recognition of the scientific basis of the technology. Expeditious approval of new products of agri-biotechnology benefits not only the USA but the global community.

Brazil is the second largest in terms of area planting under GM crops globally at 50.2 million hectares with an increase of 2% (1.1 million hectares) over 2016. It represents 26% of global GM area of 189.8 million hectares. The GM crops planted in the country included ~33.7 million hectares soybean; 15.6 million hectares of maize (summer and winter); and 940,000 hectares of cotton. The 50.2 million hectares of GM crop area is a 94% adoption rate. The area grown to GM soybean and cotton increased significantly in 2017 compared to 2016 due to profitability, higher prices, high market demand both domestically and internationally and available seed technologies. Various GM crops in pipeline include sugarcane, potato, papaya, rice and citrus. New GM products such as edible beans, eucalyptus and recently approved sugarcane will be available by 2019/2020.

Argentina maintained its ranking as the third largest producer of GM crops in the world in 2017, after the USA and Brazil occupying 12% of global hectarage (23.6 million hectares) under planting. It is one of the leading exporters of GM soybean, cotton and maize.

Canada is fourth in world ranking of GM crops. Canada planted six GM crops in 2017 at 13.12 million hectares which is an unprecedented increase of 18% from 11.1 million hectares in 2016. The four GM crops grown in Canada in 2017 were canola (8.83 million hectares), soybean (2.50 million hectares), maize (1.78 million hectares), sugar beet (15,000 hectares) with 100% adoption, alfalfa (3000 hectares) and 40 hectares of potato. The average adoption rate for the four major crops viz., soybean, maize, canola and sugar beet was at 95%. Large increase in GM crop area was obtained for reduced lignin alfalfa, HT soybean and sugar beet. GM apple will be in the consumer market in the near future.

India achieved a great stride in cotton production with a quarter of market share in global cotton production in 2017. GM cotton area increased by 6% from 10.8 million hectares in 2016 to 11.4 million hectares in 2017, equivalent to 93% of total cotton area of 12.24 million hectares. IR Bt technology in hybrids delivered broad based benefits by saving losses caused by American bollworm and boosting cotton yield to 500 kg lint per hectare. However, the next level of cotton yield target to achieve a yield level equal to the global average cotton yield of ~700 kg lint per hectare, cannot be achieved without the introduction of new GM traits including stacked traits, smart agronomy and high yielding cotton cultivars. Stewardship

and resistance management strategies need to be implemented rigorously to maintain current yield levels in existing IR cotton hybrids. IR chickpea and pigeonpea approved for field trials by the government regulatory agency in 2016.

Ten countries in Latin America grew 79.4 million hectares of GM crops, led by Brazil (50.2 million hectares), Argentina (23.6 million hectares), Paraguay (2.96 million hectares), Uruguay (1.14 million hectares), Bolivia (1.3 million hectares), Mexico (110,000 hectares), Colombia (95,000 hectares), Honduras (32,000 hectares), Chile (13,000 hectares) and Costa Rica (275 hectares) which is equal to 42% of the total global GM area. The 79.4 million hectares is a marginal decline of 110,000 hectares GM crops planted in 2016 in Latin America. This decline in GM area had been mainly due to water stresses (drought and flooding), low prices of specific commodities and local and international trade issues. Increase in GM crop areas in Chile (23%), Costa Rica (22%), Mexico (13%), Colombia (7%), Honduras (3%), and Brazil (2%) was due to profitability, higher prices, increased market demand both domestically and internationally, and presence of available seed technologies in the country. Future expansion of the major GM crops: soybean, maize and cotton may come with the increasing domestic and global demand for protein for food and animal feed, biofuel production (biodiesel for soybean and ethanol for maize) and raw cotton material.

New GM crops which are likely to be adopted by particular countries in the future are maize and sugarcane for Bolivia, maize and resumption of soybean planting for Mexico, and soybean for Honduras. The estimated over half a million farmers in the developing countries of Latin America were benefitted in the last 21 years of commercialization of GM crops. Economic benefits estimated by Brookes and Barfoot (2018) from respective country's start year of planting till 2016, was over US$46.9 billion and for 2016 alone, was about US$6.5 billion. These are enormous benefits that can only be derived from GM crops.

Eight countries in Asia and the Pacific grew 19.1 million hectares of GM crops. GM countries in the Asia and Pacific region were led by India with the biggest area of GM crops at 11.4 million hectares of cotton followed by Pakistan (3 million hectares cotton), China (2.78 million hectares cotton), Australia (9.24 million hectares cotton and canola), the Philippines (6.42 million hectares maize), Myanmar (3.20 million hectares cotton), Vietnam (45,000 hectares maize) and Bangladesh (2,400 hectares eggplant). This region planted 19.11 million hectares of GM crops, 10% of the global GM crops of 189.8 million hectares. There was an overall increase in GM crop area of 3.34% due to increase in GM cotton area in India (6%) and Pakistan (3.4%); Australia (8%) for GM cotton and canola; Vietnam (29%) for GM maize; and most notably Bangladesh (242%) for GM eggplant. Increase in GM crop areas in these countries were mainly due to farmers' acceptance of the technology because of the savings on insecticide application and labour cost for India, Pakistan, Vietnam and Bangladesh; clear

regulatory guidelines and new GM cotton varieties available in Pakistan and Myanmar; and favourable weather and increasing global demand for canola in Australia.

The expansion of GM crops in Asia and the Pacific Region depends on a number of factors specific to each country. GM cotton-growing countries viz., India, Pakistan, China, and Myanmar have various new GM cotton varieties in the pipeline requiring regulatory approvals, as well as various crops and traits. In Myanmar, GM crop regulation needs to be put in place to expedite approval and commercialization of new GM cotton varieties and other crops/traits. GM research in China has produced various GM crops with important agronomic traits including IR rice, phytase maize, HT cotton, HT soybean and many others. Over 15 million GM farmers in the developing countries of Asia have been benefiting immensely in the last 21 years of commercialization. Economic benefits estimated by Brookes and Barfoot (2018) from respective country's start year of planting till 2016 was over US$47.8 billion and for 2016 alone, by about US$3.2 billion.

Bangladesh increased its Bt eggplant planting to 700 hectares and more brinjal varieties with Bt gene are being field tested for future commercialization. In China, food and manufacturing industry considered potato as the fourth staple food with renewed interest on its research, development and production. The upcoming GM potatoes which are non-bruising, low acrylamide, lowered reducing sugar and late blight resistant, as well as beta-carotene enriched Golden Rice will help to address the issue of malnutrition and hunger in Asia and the Pacific.

Two countries in the European Union continued to plant GM maize at more than 131,000 hectares. Two countries, Spain and Portugal in the European Union have consistently planted IR maize event MON810, the only GM event approved in the EU. The total GM crop area planted was 131,535 hectares, a slight decrease of 4% from 2016. Spain planted 124,227 hectares and Portugal at 7,308 hectares. Czech Republic and Slovakia have stopped planting in 2017 due to difficulty in marketing GM maize.

South Africa and Sudan had increased planting of GM crops which has reached to 2.9 million hectares, an increase of 4% grown in 2016. South Africa, one of the top 10 countries planting >1 million hectares in 2017, continued to lead the adoption of GM crops in the African continent. The new key GM crops under advanced multi-location trials nearing commercialization for food security are banana, cassava and cowpea. Africa currently has 12 GM crops in 13 countries and 14 traits under different stages of planting, experimentation and research. Stacked traits are gaining popularity with more countries (Mozambique and Tanzania) opting for their introduction. These benefits were estimated to be US$2.5 billion from 1996 to 2016 and US$330 million in 2016 alone (Brookes and Barfoot, 2018),

The acceptance is emerging in the continent. Three countries viz., Kenya, Malawi and Nigeria transitioned from research to granting environmental release approvals, while six others viz., Burkina Faso, Ethiopia, Ghana, Nigeria, Swaziland and Uganda made significant progress in moving towards completion of multi-location trials in readiness for considering commercial approval. Three of these crops – banana, cowpea and sorghum are new and primarily for food security. Tanzania planted its first ever confined field trial of drought tolerant maize while Mozambique granted its first ever approval for a confined field trial of a stacked trait, an IR and drought tolerant maize (ISAAA, 2016).

2.1. New GM Crops and Traits in the Pipeline

Also, new GM crops and traits are being field tested to cater to farmers and consumers. These include staple crops such as beta-carotene enriched Golden Rice being tested in the Philippines and Bangladesh; *Banana bunchy top virus* resistant GM banana in Uganda; *Fusarium* wilt resistant GM banana and GM wheat with disease resistance, drought tolerance, altered oil content and grain composition in Australia; high yield and biomass wheat in the UK; late blight resistant potato varieties Desiree and Victoria in Uganda and late blight and nematode resistant potato variety Maris Piper with less bruising and less acrylamide potato in the EU; IR chickpea and pigeonpea in India; drought tolerant sugarcane in India and Indonesia; and omega-3 enriched camelina in the EU (ISAAA, 2016).

3. Status of Approved Events for GM Crops Used as Food, Feed and for Processing

GM crops were planted in small scale as early as 1994 and large scale plantings were recorded in 1996. There have been 4,133 approvals granted by regulatory authorities of 67 countries (39+EU 28) to GM crops for consumption either as food and/or feed as well as for environmental release. These were granted to 476 GM events from 26 GM crops, excluding carnation, rose and petunia. Of these approvals, 1,995 are for food, either for direct use or for processing, 1,338 are for feed use (direct use or for processing) and 800 are for environmental release or cultivation. Japan has the largest number of GM events approved (not including the intermediate events from approved stacked and pyramided events), followed by USA, Canada, South Korea etc. (Table 2) (ISAAA, 2017).

Table 2. Number of approved events for food, feed and cultivation/environment in top ten countries*

Rank	Country	Food	Feed	Cultivation	Total
1.	Japan*	295	197	154***	646
2.	USA**	185	179	175	539
3.	Canada	141	136	142	419
4.	South Korea	148	140	0	288
5.	European Union	97	97	10	204
6.	Brazil	76	76	76	228
7.	Mexico	170	5	15	190
8.	Philippines	88	87	13	188
9.	Argentina	61	60	60	181
10.	Australia	112	15	48	175
11.	Others	622	346	107	1075
	Total	**1,995**	**1,338**	**800**	**4,133**

* For Japan, data is collected from Japan Biosafety Clearing-House and the website of the Ministry of Health, Labour and Welfare. However, intermediate events derived from an approved pyramided event recorded in JBCH

** USA only approves individual events

*** While cultivation approvals are granted in Japan, there is no current GM planting done.

Source: ISAAA (2017)

Maize still has the most number of approved events (231 in 30 countries), followed by cotton (60 events in 24 countries), potato (48 events in 10 countries), canola (41 events in 15 countries) and soybean (40 events in 29 countries). The HT maize event NK603 (55 approvals in 26 countries + EU-28) still has the most number of approvals. It is followed by HT soybean GTS 40-3-2 (54 approvals in 27 countries + EU-28), IR maize MON810 (53 approvals in 26 countries + EU-28), IR maize Bt11 (51 approvals in 25 countries + EU-28), IR

maize TC1507 (51 approvals in 24 countries + EU-28), HT maize GA21 (50 approvals in 24 countries + EU-28), IR maize MON89034 (49 approvals in 24 countries + EU-28), HT soybean A2704-12 (43 approvals in 23 countries + EU-28), IR maize MON88017 (42 approvals in 22 countries + EU-28), IR cotton MON531 (43 approval in 21 countries + EU-28), herbicide tolerant maize T25 (41 approvals in 20 countries + EU-28) and IR maize MIR162 (41approvals in 21 countries + EU-28). Crop-wise number of approved events for food, feed and cultivation are given in Table 3 (http://www.isaaa.org/gmapprovaldatabase/).

Table 3. Crop-wise number of approved events for food, feed and cultivation

Crop	Total number of events	Number of events approved for		
		Food	Feed	Cultivation
Alfalfa	5	5	5	5
Apple	3	3	3	3
Canola, *Brassica napus*	41	41	41	31
Canola, *B. rapa*	4	-	4	4
Cotton	60	50	48	53
Eggplant	1	1	-	1
Maize	231	227	151	112
Papaya	4	2	2	4
Potato	48	47	44	35
Soybean	40	38	34	35
Squash	2	2	2	2
Sugar beet	3	3	3	3

The crop-wise information on the number of events approved in different countries for use directly as food / processing; feed/ processing or for cultivation for domestic/ non-domestic (Table 4) use has been compiled from the various databases available at http://www.isaaa.org/gmapprovaldatabase; http://www.isaaa.org/gmapprovaldatabase/eventslist/default;asp;

http://www.isaaa.org/gmapprovaldatabase/crop/default.asp?CropID=6&Crop;
http://www.isaaa.org/gmapprovaldatabase/approvedeventsin default.asp?CountryID=AR&Country=

Table 4. Crop-wise number of approved events in different countries

Country	Crop	Total no. of events	No. of events approved for		
			Food direct use/processing	Feed direct use/processing	Cultivation domestic/ non-domestic use
USA	Alfalfa	3	3	3	3
	Apple	3	3	3	3
	Canola	21	20	19	16
	Cotton	28	27	26	25
	Maize	43	41	41	42
	Papaya	3	2	2	3
	Potato	43	43	43	34
	Soybean	25	21	20	24
	Squash	2	2	2	2
	Sugar beet	3	3	3	3
Brazil	Cotton	15	15	15	15
	Maize	44	44	44	44
	Soybean	17	17	17	15
Argentina	Cotton	7	6	6	7
	Maize	45	45	44	45
	Soybean	15	15	15	14
Canada	Alfalfa	3	3	3	3
	Canola	19 (*Brassica napus*) 4 (*B. rapa*)	19 -	19 4	19 4
	Maize	67	39	38	66
	Soybean	21	19	19	21

	Sugar beet	**2**	**2**	**2**	**2**
India	Cotton	5	-	-	**5**
Paraguay	Cotton	4	4	4	4
	Maize	15	13	13	15
	Soybean	3	2	1	3
Pakistan	Cotton	2	-	-	2
China	Cotton	11	8	9	2
	Papaya	1	-	-	1
South	Cotton	10	6	6	9
Africa	Maize	42	41	41	11
	Soybean	12	11	11	1
Uruguay	Maize	10	9	7	10
	Soybean	7	2	2	7
Bolivia	Soybean	1	1	1	1
Australia	Canola	23	23	10	21
	Cotton	27	27	7	16
Philippines	Maize	52	52	51	14
Myanmar	Cotton	1	-	-	1
Spain	Maize	-	-	-	-
Sudan	Cotton	1	-	-	1
Mexico	Cotton	31	31	5	12
	Soybean	26	26	-	1
Colombia	Cotton	14	12	7	6
	Maize	49	49	31	6
Vietnam	Maize	14	14	14	4

Honduras	Maize	7	2	1	5
Chile	Maize	1	-	-	1
Portugal	Maize		-		
Bangladesh	Brinjal/ eggplant	1	1	-	1
Costa Rica	Cotton Pineapple	17 -	-	-	17

Following the Cartagena Protocol on Biosafety, countries allow entry of only approved GM events. Some countries have stringent or long process of approvals that cause problems if imported products contain unapproved events, especially in a stacked event. India has operationalized event based approval mechanism. The Ministry of Environment, Forest and Climate Change (MoEF&CC) has notified a new procedure for commercial release of Bt cotton hybrids expressing approved events called "Event Based Approval Mechanism (EBAM)" (http://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?id=3900). This mechanism will be applicable to new cotton hybrids expressing five approved events. The approval mechanism which is initially applicable to approved cotton events, will speed up the introduction of new GM crops to the country without compromising biosafety including environmental safety. More information about the "New Procedure for Commercial Release of Bt Cotton Hybrids Expressing Approved Events" is available at http://www.envfor.nic.in/divisions/csurv/geac/New%20procedure%20under%20EABM.pdf. (ISAAA, 2009).

The continuing immense growth of GM crop adoption for cultivation and import globally, is a manifestation of farmer and consumer satisfaction with the agricultural, socio-economic, and environmental benefits as well as food safety and nutritional improvement brought by GM crops. Thus, improvements in modern crop technology and agronomic practices have to be fully utilized because they have the capacity to reduce annual fluctuations in food availability as well as maintain nutritive contents of crops. Both mitigation and adaptation technologies are crucial in combating climate change. Adoption of GM crops is one of the most effective crop adaptation technologies to combat climate change because crop varieties may be developed in a timely manner through modern methods of molecular biology and biotechnology to cope up with salinity, submergence and drought, as well as more virulent newly emergent insect pests and plant pathogens.

The technology in conjunction with conducive policies can double food production. Finally, GM technology will continue benefiting the burgeoning population with new GM

crops and traits to cater to the needs of farmers and consumers. However, even after 22 years of successful commercialization of GM crops, there are some challenges including the stringent regulatory mechanism that limits scientific innovation and restricts technology development that would have benefited farmers and consumers.

4. References

- Brookes G and Barfoot P (2018) GM crops: Global socio-economic and environmental impacts 1996-2017. http://biotechbenefits.croplife.org/paper/gm-crops-global-socio-economic-and-environmental-impacts. Retrieved July 5, 2018.
- http://www.isaaa.org/gmapprovaldatabase. Retrieved July 5, 2018.
- http://www.isaaa.org/gmapprovaldatabase/approvedeventsin/default.asp?CountryID=AR&Country. Retrieved July 5, 2018.
- http://www.isaaa.org/gmapprovaldatabase/crop/default.asp?CropID=6&Crop. Retrieved July 5, 2018.
- http://www.isaaa.org/gmapprovaldatabase/eventslist/default.asp. Retrieved July 5, 2018.
- http://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?id=3900. Retrieved July 5, 2018.
- ISAAA (2009) New procedure for commercial release of Bt cotton hybrids expressing approved events, Crop Biotech Update March 27, 2009 issue. http://www.envfor.nic.in/divisions/csurv/geac/New%20procedure%20under%20EABM.pdf.
- ISAAA (2016) *Global Status of Commercialized Biotech/GM Crops* . ISAAA Brief No. 52. ISAAA: Ithaca, NY. http://www.isaaa.org/. Retrieved June 10, 2018.
- ISAAA (2017) *Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years* . ISAAA Brief No. 53. ISAAA: Ithaca, NY. http://www.isaaa.org/. Retrieved June 28, 2018.

Cartagena Protocol on Biosafety: An Overview

3

Chimata Murali Krishna

Ministry of Environment, Forest and Climate Change, Govt. of India
New Delhi 110003, India
Email: *cm.krishna@gov.in*

1. Introduction

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD) is an international treaty governing the movement of living modified organisms (LMOs) resulting from modern biotechnology from one country to another. The Protocol seeks to protect biological diversity from the potential risks posed by LMOs resulting from modern biotechnology. It establishes an advance informed agreement (AIA) procedure for ensuring that countries are provided with the information necessary to make informed decisions before agreeing to the import of such organisms into their territory. The Protocol contains reference to a precautionary approach and reaffirms the precaution language in Principle 15 of the Rio Declaration on Environment and Development. The Protocol also establishes a Biosafety Clearing-House (BCH) to facilitate the exchange of information on LMOs and to assist countries in the implementation of the Protocol. It was adopted on 29 January 2000 as a supplementary agreement to the CBD and entered into force on 11 September 2003. As on June 2018, 171 countries have ratified this Protocol and India is also a Party to this Protocol since 2003.

The objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of LMOs resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.

The Protocol has a Preamble and 40 Articles along with three Annexures as detailed below:

Preamble

Article 1	Objective
Article 2	General Provisions
Article 3	Use of Terms
Article 4	Scope
Article 5	Pharmaceuticals
Article 6	Transit and Contained Use
Article 7	Application of the Advance Informed Agreement Procedure
Article 8	Notification
Article 9	Acknowledgement of Receipt of Notification
Article 10	Decision Procedure
Article 11	Procedure for Living Modified Organisms Intended for Direct Use as Food or Feed, or for Processing
Article 12	Review of Decisions
Article 13	Simplified Procedure
Article 14	Bilateral, Regional and Multilateral Agreements and Arrangements
Article 15	Risk Assessment
Article 16	Risk Management
Article 17	Unintentional Transboundary Movements and Emergency Measures
Article 18	Handling, Transport, Packaging and Identification
Article 19	Competent National Authorities and National Focal Points
Article 20	Information Sharing and the Biosafety Clearing-House

Article 21	Confidential Information
Article 22	Capacity-Building
Article 23	Public Awareness and Participation
Article 24	Non-Parties
Article 25	Illegal Transboundary Movements
Article 26	Socio-Economic Considerations
Article 27	Liability and Redress
Article 28	Financial Mechanism and Resources
Article 29	Conference of the Parties Serving as the Meeting of the Parties to this Protocol
Article 30	Subsidiary Bodies
Article 31	Secretariat
Article 32	Relationship with the Convention
Article 33	Monitoring and Reporting
Article 34	Compliance
Article 35	Assessment and Review
Article 36	Signature
Article 37	Entry Into Force
Article 38	Reservations
Article 39	Withdrawal
Article 40	Authentic Texts
Annex I	Information required in notifications under articles 8, 10 and 13
Annex II	Information required concerning living modified organisms intended for direct use as food or feed, or for processing under article 11
Annex III	Risk Assessment

2. Key Features of Some of the Important Articles

2.1. Scope (Article 4)

This Protocol shall apply to the transboundary movement, transit, handling and use of all LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

2.2. Pharmaceuticals (Article 5)

This Protocol shall not apply to the transboundary movement of LMOs which are pharmaceuticals for humans that are addressed by other relevant international agreements or organisations.

2.3. Transit and Contained Use (Article 6)

The advance informed agreement (AIA) procedure shall not apply to LMOs in transit and also to the transboundary movement of LMOs destined for contained use undertaken in accordance with the standards of the Party of import.

2.4. Advance Informed Agreement Procedure (Article 7)

The AIA procedure shall apply prior to the first intentional transboundary movement of LMOs for intentional introduction into the environment of the Party of import and Article 11 shall apply prior to the first transboundary movement of LMOs intended for direct use as food or feed, or for processing.

The AIA procedure shall not apply to the intentional transboundary movement of LMOs identified in a decision of the Conference of the Parties serving as the meeting of the Parties to this Protocol as being not likely to have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

2.5. Notification (Article 8)

The Party of export shall notify, or require the exporter to ensure notification to, in writing, the competent national authority of the Party of import prior to the intentional transboundary movement of a LMO. The notification shall contain, at a minimum, the information specified in Annex I. The Party of export shall ensure that there is a legal requirement for the accuracy of information provided by the exporter.

2.6. Acknowledgement of Receipt of Notification (Article 9)

The Party of import shall acknowledge receipt of the notification, in writing, to the notifier

within 90 days of its receipt and it would acknowledge the date of receipt of the notification; whether the notification, prima facie, contains the information referred to in Article 8; whether to proceed according to the domestic regulatory framework of the Party of import or according to the procedure specified in Article 10.

A failure by the Party of import to acknowledge receipt of a notification shall not imply its consent to an intentional transboundary movement.

2.7. Decision Procedure (Article 10)

- The Party of import shall, within the period of time referred to in Article 9, inform the notifier, in writing, whether the intentional transboundary movement may proceed:
  - Only after the Party of import has given its written consent; or
  - After no less than 90 days without a subsequent written consent.
- Within 270 days of the date of receipt of notification, the Party of import shall communicate, in writing, to the notifier and to the BCH the decision, whether the Party is:
  - Approving the import, with or without conditions, including how the decision will apply to subsequent imports of the same LMO;
  - Prohibiting the import;
  - Requesting additional relevant information in accordance with its domestic regulatory framework or Annex I; in calculating the time within which the Party of import is to respond, the number of days it has to wait for additional relevant information shall not be taken into account; or
  - Informing the notifier that the period specified in this paragraph is extended by a defined period of time.
- Except in a case in which consent is unconditional, a decision shall set out the reasons on which it is based.
- A failure by the Party of import to communicate its decision within 270 days of the date of receipt of the notification shall not imply its consent to an intentional transboundary movement.
- Further, lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a LMO on the

conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of the LMO in question as above, in order to avoid or minimize such potential adverse effects.

2.8. Procedure for LMOs Intended for Direct Use as Food or Feed, or for Processing (Article 11)

- A Party that makes a final decision regarding domestic use, including placing on the market, of a LMO that may be subjected to transboundary movement for direct use as food or feed, or for processing shall, within 15 days of making that decision, inform the Parties through the BCH. This information shall contain, at a minimum, the information specified in Annex II. The Party shall provide a copy of the information, in writing, to the national focal point of each Party that informs the Secretariat in advance that it does not have access to the BCH. This provision shall not apply to decisions regarding field trials.

- The Party making a decision shall ensure that there is a legal requirement for the accuracy of information provided by the applicant and any Party may request additional information from the authority identified in Annex II.

- A Party may take a decision on the import of LMOs intended for direct use as food or feed, or for processing, under its domestic regulatory framework that is consistent with the objective of this Protocol.

- Each Party shall make available to the BCH copies of any national laws, regulations and guidelines applicable to the import of LMOs intended for direct use as food or feed, or for processing, if available.

- A developing country Party or a Party with an economy in transition may, in the absence of the domestic regulatory framework, and in exercise of its domestic jurisdiction, declare through the BCH that its decision prior to the first import of a LMO intended for direct use as food or feed, or for processing, on which information has been provided will be taken according to the following:

    - A risk assessment undertaken in accordance with Annex III; and

    - A decision made within a predictable time frame, not exceeding two hundred and seventy days.

- Failure by a Party to communicate its decision shall not imply its consent or refusal to

the import of a LMO intended for direct use as food or feed, or for processing, unless otherwise specified by the Party.

- Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a LMO on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of that LMO intended for direct use as food or feed, or for processing, in order to avoid or minimize such potential adverse effects.

2.9. Review of Decisions (Article 12)

- A Party of import may, at any time, in light of new scientific information on potential adverse effects on the conservation and sustainable use of biological diversity, taking also into account the risks to human health, review and change a decision regarding an intentional transboundary movement. In such case, the Party shall, within 30 days, inform any notifier that has previously notified movements of the LMO referred to in such decision, as well as the BCH, and shall set out the reasons for its decision.

- A Party of export or a notifier may request the Party of import to review a decision it has made in respect of it under Article 10 where the Party of export or the notifier considers that:

    - A change in circumstances has occurred that may influence the outcome of the risk assessment upon which the decision was based; or

    - Additional relevant scientific or technical information has become available.

- The Party of import shall respond in writing to such a request within 90 days and set out the reasons for its decision.

- The Party of import may, at its discretion, require a risk assessment for subsequent imports.

2.10. Risk Assessment (Article 15)

- Risk assessments undertaken pursuant to this Protocol shall be carried out in a scientifically sound manner, in accordance with Annex III and taking into account recognized risk assessment techniques. Such risk assessments shall be based, at a minimum, on information provided in accordance with Article 8 and other available scientific evidence in order to identify and evaluate the possible adverse effects of LMOs

on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

- The Party of import shall ensure that risk assessments are carried out for decisions taken under Article 10. It may require the exporter to carry out the risk assessment. The cost of risk assessment shall be borne by the notifier if the Party of import so requires.

2.11. Risk Management (Article 16)

- The Parties shall, taking into account Article 8 (g) of the CBD, establish and maintain appropriate mechanisms, measures and strategies to regulate, manage and control risks identified in the risk assessment provisions of this Protocol associated with the use, handling and transboundary movement of LMOs.

- Measures based on risk assessment shall be imposed to the extent necessary to prevent adverse effects of the LMO on the conservation and sustainable use of biological diversity, taking also into account risks to human health, within the territory of the Party of import.

- Each Party shall take appropriate measures to prevent unintentional transboundary movements of LMOs, including such measures as requiring a risk assessment to be carried out prior to the first release of a LMO.

- Each Party shall endeavour to ensure that any LMO, whether imported or locally developed, has undergone an appropriate period of observation that is commensurate with its life cycle or generation time before it is put to its intended use.

- Parties shall cooperate with a view to identify LMOs or specific traits of LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health; and taking appropriate measures regarding the treatment of such LMOs or specific traits.

2.12. Unintentional Transboundary Movements and Emergency Measures (Article 17)

Each Party shall take appropriate measures to notify affected or potentially affected States, the BCH and, where appropriate, relevant international organisations, when it knows of an occurrence under its jurisdiction resulting in a release that leads, or may lead, to an unintentional transboundary movement of a LMO that is likely to have significant adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health in such States. The notification shall be provided as soon as the Party knows of the above situation and also to provide available relevant information on the estimated quantities and relevant characteristics and/or traits of the LMO; information on

the circumstances and estimated date of the release, and on the use of the LMO in the originating Party; any available information about the possible adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, as well as available information about possible risk management measures; and a point of contact for further information.

Each Party shall, no later than the date of entry into force of this Protocol for it, make available to the BCH the relevant details setting out its point of contact for the purposes of receiving notifications under this Article.

In order to minimize any significant adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, each Party, under whose jurisdiction the release of the LMO occurs, shall immediately consult the affected or potentially affected States to enable them to determine appropriate responses and initiate necessary action, including emergency measures.

2.13. Handling, Transport, Packaging and Identification (Article18)

In order to avoid adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, each Party shall take necessary measures to require that LMOs that are subject to intentional transboundary movement within the scope of this Protocol are handled, packaged and transported under conditions of safety, taking into consideration relevant international rules and standards.

Each Party shall take measures to require that documentation accompanying:

- LMOs that are intended for direct use as food or feed, or for processing, clearly identifies that they "may contain" LMOs and are not intended for intentional introduction into the environment, as well as a contact point for further information. The Conference of the Parties serving as the meeting of the Parties to this Protocol (COP-MOP) shall take a decision on the detailed requirements for this purpose, including specification of their identity and any unique identification, no later than two years after the date of entry into force of this Protocol;

- LMOs that are destined for contained use clearly identifies them as LMOs and specifies any requirements for the safe handling, storage, transport and use, the contact point for further information, including the name and address of the individual and institution to whom the LMOs are consigned; and

- LMOs that are intended for intentional introduction into the environment of the Party of import and any other LMOs within the scope of the Protocol, clearly identifies them as

LMOs; specifies the identity and relevant traits and/or characteristics, any requirements for the safe handling, storage, transport and use, the contact point for further information and, as appropriate, the name and address of the importer and exporter; and contains a declaration that the movement is in conformity with the requirements of this Protocol applicable to the exporter.

2.14. Competent National Authorities and National Focal Points (Article 19)

Each Party shall designate one national focal point (NFP) to be responsible on its behalf for liaison with the Secretariat. Each Party shall also designate one or more competent national authorities (CNAs), which shall be responsible for performing the administrative functions required by this Protocol and which shall be authorized to act on its behalf with respect to those functions. A Party may designate a single entity to fulfil the functions of both NFP and CNA.

2.15. Information Sharing and the Biosafety Clearing-House (Article 20)

- BCH is hereby established as part of the clearing-house mechanism under Article 18, paragraph 3, of the Convention, in order to:
    - Facilitate the exchange of scientific, technical, environmental and legal information on, and experience with, LMOs
    - Assist Parties to implement the Protocol, taking into account the special needs of developing country Parties, in particular the least developed and small island developing States among them, and countries with economies in transition as well as countries that are centres of origin and centres of genetic diversity.
- The BCH shall serve as a means through which information is made available for the purposes of paragraph 1 above. It shall provide access to information made available by the Parties relevant to the implementation of the Protocol. It shall also provide access, where possible, to other international biosafety information exchange mechanisms.
- Without prejudice to the protection of confidential information, each Party shall make available to the BCH any information required to be made available to the BCH under this Protocol, and:
    - Any existing laws, regulations and guidelines for implementation of the Protocol, as well as information required by the Parties for the AIA procedure;
    - Any bilateral, regional and multilateral agreements and arrangements;

- Summaries of its risk assessments or environmental reviews of LMO generated by its regulatory process, and carried out in accordance with Article 15, including, where appropriate, relevant information regarding products thereof, namely, processed materials that are of LMO origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology;

- Its final decisions regarding the importation or release of LMOs; and

- Reports submitted by it pursuant to Article 33, including those on implementation of the AIA procedure.

2.16. Capacity-Building (Article 22)

- Each Party shall cooperate in the development and/or strengthening of human resources and institutional capacities in biosafety, including biotechnology to the extent that it is required for biosafety, for the purpose of the effective implementation of this Protocol, in developing country Parties, in particular the least developed and small island developing States among them, and in Parties with economies in transition, including through existing global, regional, sub-regional and national institutions and organisations and, as appropriate, through facilitating private sector involvement.

- For the purpose of implementing capacity building activities, the needs of developing country Parties, in particular the least developed and small island developing States among them, for financial resources and access to and transfer of technology and know-how in accordance with the relevant provisions of the CBD, shall be taken fully into account for capacity-building in biosafety.

- Cooperation in capacity-building shall, subject to the different situations, capabilities and requirements of each Party, include scientific and technical training in the proper and safe management of biotechnology, and in the use of risk assessment and risk management for biosafety, and the enhancement of technological and institutional capacities in biosafety. The needs of Parties with economies in transition shall also be taken fully into account for such capacity-building in biosafety.

2.17. Public Awareness and Participation (Article 23)

Each Party shall

- Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of LMOs in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health.

- Endeavour to ensure that public awareness and education encompass access to information on LMOs identified in accordance with this Protocol that may be imported.

- The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding LMOs and shall make the results of such decisions available to the public, while respecting confidential information.

- Each Party shall endeavour to inform its public about the means of public access to the BCH.

2.18. Illegal Transboundary Movements (Article 25)

- Each Party shall adopt appropriate domestic measures aimed at preventing and, if appropriate, penalizing transboundary movements of LMOs carried out in contravention of its domestic measures to implement this Protocol. Such movements shall be deemed illegal transboundary movements.

- In the case of an illegal transboundary movement, the affected Party may request the Party of origin to dispose, at its own expense, of the LMO in question by repatriation or destruction, as appropriate.

- Each Party shall make available to the BCH information concerning cases of illegal transboundary movements pertaining to it.

2.19. Socio-Economic Considerations (Article 26)

The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of LMOs on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities.

The Parties are encouraged to cooperate on research and information exchange on any socio-economic impacts of LMOs, especially on indigenous and local communities.

2.20. Liability and Redress (Article 27)

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall, at its first meeting, adopt a process with respect to the appropriate elaboration of international rules and procedures in the field of liability and redress for damage resulting from transboundary movements of LMOs, analysing and taking due account of the ongoing

processes in international law on these matters, and shall endeavour to complete this process within four years.

2.21. Conference of the Parties Serving as the Meeting of the Parties to this Protocol (Article 29)

The Conference of the Parties shall serve as the meeting of the Parties to this Protocol. Parties to the CBD that are not Parties to this Protocol may participate as observers in the proceedings of any meeting of the COP-MOP. When the Conference of the Parties serves as the meeting of the Parties to this Protocol, decisions under this Protocol shall be taken only by those that are Parties to it.

2.22. Monitoring and Reporting (Article 33)

Each Party shall monitor the implementation of its obligations under this Protocol, and shall, at intervals to be determined by the Conference of the Parties serving as the meeting of the Parties to this Protocol, report to the Conference of the Parties serving as the meeting of the Parties to this Protocol on measures that it has taken to implement the Protocol.

2.23. Assessment and Review (Article 35)

The Conference of the Parties serving as the meeting of the Parties to this Protocol shall undertake, five years after the entry into force of this Protocol and at least every five years thereafter, an evaluation of the effectiveness of the Protocol, including an assessment of its procedures and annexes.

2.24. Withdrawal (Article 39)

At any time after two years from the date on which this Protocol has entered into force for a Party, that Party may withdraw from the Protocol by giving written notification to the Depositary.

Any such withdrawal shall take place upon expiry of one year after the date of its receipt by the Depositary, or on such later date as may be specified in the notification of the withdrawal.

The Cartagena Protocol on Biosafety is the only international instrument that deals exclusively with LMOs. The other international instruments and standard-setting processes addressing other aspects of biosafety are:

- International Plant Protection Convention (IPPC) - GM plant pests

- Codex Alimentarius Commission (CAC)- GM food safety
- World Organization for Animal Health (OIE) – for health of GM animals, e.g. GM vaccines for animals
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (WTO-SPS)

The Ministry of Environment, Forest and Climate Change (MoEF & CC) is the NFP for the implementation of various provisions of the Cagena Protocol in India.

3. Reference

The Cartagena Protocol on Biosafety. http://www.biodiv.org/biosafety and https://bch.cbd.int/protocol/text/. Retrieved May 24, 2017.

Indian Biosafety Regulatory Framework for Living Modified Organisms

4

Chimata Murali Krishna

Ministry of Environment, Forest and Climate Change, Govt. of India
New Delhi 110003, India
Email: *cm.krishna@gov.in*

1. Introduction

Modern biotechnology (recombinant DNA technology) is recognized to have great potential for the promotion of human well-being, particularly in meeting critical needs for food, agriculture and health care. Recombinant DNA (r-DNA) technology, the ability to transfer genetic material through biochemical means, since the early 1970s, has enabled scientists to genetically modify plants, animals, and microorganisms rapidly, which are generally referred to as Genetically Modified Organisms (GMOs). Modern genetic engineering (GE) techniques facilitated introduction of a greater diversity of genes into organisms, including those from unrelated species, which could not have been possible through traditional methods of breeding and selection.

GMOs developed through r-DNA technology have tremendously contributed in the areas of health care, agriculture, processing industry and environmental management. However, there are also several biosafety (environmental/ health) related concerns regarding potential risks and hazards arising from the use of these GMOs and products derived from them.

Biosafety refers to protecting the environment including human and animal health from the possible adverse effects of the GMOs and the products derived from the use of modern biotechnology. To address the above concerns, biosafety regulations have been developed by various countries involved in research in transgenic crops and their commercialization.

2. Environment (Protection) Act, 1986

The Ministry of Environment and Forests, now Ministry of Environment, Forest and Climate Change (MoEF&CC) had enacted Environment (Protection) Act, 1986 (EPA) to provide for the protection and improvement of environment and the related matters. Environment includes

water, air and land and the interrelationship, which exists among and between water, air and land, and human beings, other living creatures, plants, microorganism and property. Some important sections of EPA are listed in Table 1.

Table 1. Important sections of Environment (Protection) Act, 1986

Section	Details
3	Central Government shall have the powers to take all such measures for the purpose of protecting and improving the quality of the environment and preventing, controlling and abating environmental pollution.
6	Central Government has powers to: • make rules on environmental safety issues. These include powers to maintain standards of quality of air, water and soil. • set limits of pollutants. • set procedures and safe guards for handling hazardous substances. • prohibit or restrict use in locations. • set procedures for containing / minimizing risks. • order that no person can violate the rules and procedures.
7	No person carrying on any industry, operation or process shall discharge or emit or permit to be discharged or emitted any environmental pollutants in excess of such standards as may be prescribed.
8	No person shall handle or cause to be handled any hazardous substance except in accordance with such procedure and after complying with such safeguards as may be prescribed.
15	Whoever fails to comply with or contravenes the Act or any rules can be punished with imprisonment for a term up to 5 years, or with a fine up to Rs. 100,000 or with both. If failure or contravention continues beyond one year, the offender may be punishable with imprisonment which may extend up to 7 years.
25	The Central Government can make rules for the purpose of this Act. The rules may contain standards, procedures for handling, authorities to intimate, manner of collecting samples, the intention of collecting samples, functions of authorized laboratories, qualifications of analysts, the manner of making complaints to the governments, the authority/authorities implementing the rules and related matters, and the powers of the authority/authorities for directing the generation of information in open environment.

Under this Act, "Rules for the Manufacture/Use/Import/Export and Storage of Hazardous Microorganisms, Genetically Engineered Organisms or Cells" were notified by MoEF&CC through their Notification No. 621 in Official Gazette of Govt. of India on December 5, 1989 (Rules 1989) . These rules and regulations cover the areas of research as well as large scale applications of GMOs and products made therefrom throughout India. The rules also cover the application of hazardous microorganisms which may not be genetically modified. Hazardous microorganisms include those which are pathogenic to human beings, animals as well as plants. The rules cover activities involving manufacture, use, import, export, storage and research. The target substances covered are, besides the hazardous natural microorganisms, all genetically engineered organisms (GEOs) including microorganisms, plants and animals.

The notification orders compliance of the safeguards through voluntary as well as regulatory approach and any violation and non-compliance including non-reporting of the activity in this area would attract punitive actions provided under the EPA. There are 20 paras in the Rules 1989 and some of the important paras along with relevant details are given in Table 2.

Table 2. Important paras of Rules 1989

Para	Deals with
7	Approvals to individuals on the import, export, transport, manufacture, process, use or sell of GMOs and use of GMOs for research
8	Authorisation for production of GMOs, plants and animals
9	Approval for deliberate or unintentional release of GMOs into the open environment
10 & 11	Approval for substances, which may contain GMOs
12	Procedures for obtaining approvals in different conditions
13	Conditions of approval of GMOs
14	Mechanism for supervising the implementation of terms and conditions given with authorization for commercial use
15	Penalties that can be levied for non-compliance of measures for safe use of GMOs and products thereof
19	Redressal mechanism through appellate authority

These rules also defined the competent authorities and composition of such authorities for handling of various aspects of the rules. Presently there are six Competent Authorities as per the rules, brief description of their broad responsibilities is as described below:

2.1. The Recombinant DNA Advisory Committee (RDAC)

The Recombinant DNA Advisory Committee (RDAC) constituted by the Department of Biotechnology (DBT), Ministry of Science & Technology, Govt. of India takes note of developments in biotechnology at national and international levels. The RDAC prepares recommendations from time to time that are suitable for implementation for upholding the safety regulations in research and applications of GMOs and products thereof.

2.2. Institutional Biosafety Committee (IBSC)

The Institutional Biosafety Committee (IBSC) is the nodal point for interaction within the institution for implementation of the guidelines. As such, in the first instance, it is necessary that the institutions intending to carry out research activities involving genetic manipulation of microorganisms, plants or animals should constitute the IBSC. All the IBSCs have to induct one DBT nominee. The main activities of IBSCs are:

- To note and to approve r-DNA work.
- To ensure adherence of r-DNA safety guidelines of government.
- To prepare emergency plan according to guidelines.
- To recommend to the Review Committee on Genetic Manipulation (RCGM) about category III risk or above experiments and to seek RCGM's approval.
- To inform District Level Committee and State Biotechnology Coordination Committee as well as Genetic Engineering Appraisal Committee about the experiments where ever needed.
- To act as nodal point for interaction with statutory bodies.
- To ensure experimentation at designated location, taking into account approved protocols.

2.3. Review Committee on Genetic Manipulation (RCGM)

The RCGM under the DBT has the following functions:

- To bring out manuals of guidelines specifying procedures for regulatory process on GMOs

in research, use and applications including industry with a view to ensure environmental safety.

- To review all on going r-DNA projects involving high risk category and controlled field experiments.
- To lay down procedures for restriction or prohibition, production, sale, import and use of GMOs both for research and applications.
- To authorize imports of GMOs/ transgenes for research purposes.

2.4. Genetic Engineering Appraisal Committee (GEAC)

Genetic Engineering Appraisal Committee (GEAC), formerly Genetic Engineering Approval Committee functions as a body under the MoEF&CC is responsible for approval of activities involving large scale use of hazardous microorganisms and recombinant products in research and industrial production from the environment angle.

- To permit the use of GMOs and products thereof for commercial applications.
- To adopt procedures for restriction or prohibition, production, sale, import and use of GMOs both for research and applications under EPA.
- To authorize large scale production and release of GMOs and products thereof into the environment.
- To authorize agencies or persons to have powers to take punitive actions under the EPA.

2.5. State Biotechnology Coordination Committee (SBCC)

This Committee, headed by the Chief Secretary of the State is constituted in each state where research and applications of GMOs are contemplated. It has:

- Powers to inspect, investigate and to take punitive action in case of violations of statutory provisions through the State Pollution Control Board or the Directorate of Health etc.
- To review periodically the safety and control measures in various institutions handling GMOs.
- To act as nodal agency at State level to assess the damage, if any, due to release of GMOs and to take on site control measures.

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.

2.6. District Level Committee (DLC)

This Committee constituted at the district level is considered to be smallest authoritative unit to monitor the safety regulations in institutions 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 institutions with a view to ensure the implementation of safety guidelines while handling GMOs, under the Indian EPA. Its functions are:

- To monitor the implementation of safety regulations.
- Has powers to inspect, investigate and report to the SBCC or the GEAC about compliance or non-compliance of r-DNA guidelines or violations under EPA.
- To act as nodal agency at District level to assess the damage, if any, due to release of GMOs and to take on site control measures.

The roles of various committees and Ministries are summarized in Table 3 and Table 4.

Table 3. Role of Statutory Committees notified under Rules 1989

Statutory committee	Function	Administrating agency
Genetic Engineering Appraisal Committee (GEAC)	Regulatory	Ministry of Environment, Forest and Climate Change
Recombinant DNA Advisory Committee (RDAC)	Advisory	Department of Biotechnology, Ministry of Science & Technology
Review Committee on Genetic Manipulation (RCGM)	Regulatory	
Institutional Biosafety Committee (IBSC)	Regulatory	Registered Institutions, Universities and Private Companies
State Biotechnology Coordination Committee (SBCC)	Monitoring	Concerned State Governments
District Level Committee (DLC)	Monitoring	

Table 4. Role of various Ministries in review, approval and monitoring of GE crops

Department/Ministry	Role of Ministry
Ministry of Environment, Forest and Climate Change	• Primarily responsible for conservation and protection of environment, ensuring environmental and human health safety before release of LMOs
Department of Biotechnology, Ministry of Science and Technology	• Promotion of biotechnology • Provide services in areas of research, infrastructure, generation of human resource
Ministry of Agriculture and Farmers' Welfare	• Policies aimed at agriculture growth • ICAR responsible for monitoring agronomic benefits of GE technology • Post-release performance of GE crops
Ministry of Health and Family Welfare	• Policies aimed at protecting and monitoring human health
Department of Customs, Ministry of Commerce and Industry	• Enhance trade with other countries through export/import policies • Enforcement at point of entry

3. Approval and Prohibitions under Rules 1989 can be summarized as below:

- No person shall import, export, transport, manufacture, process, use or sell any GMOs, substances or cells except with the approval of the GEAC.
- Use of pathogenic organisms or GMOs or cells for research purpose shall be allowed under the Notification, 1989 of the EPA, 1986.
- Any person operating or using GMOs for scale up or pilot operations shall have to obtain permission from GEAC.
- For purpose of education, experiments on GMOs, IBSC can look after, as per the guidelines of the Government of India.
- Deliberate or unintentional release of GMOs not allowed.
- Production in which GMOs are generated or used shall not be commenced except with the approval of GEAC

- GEAC supervises the implementation of rules and guidelines.
- GEAC carries out supervision through SBCC, DLC or any authorized person.
- If orders are not complied, SBCC/DLC may take suitable measures at the expense of the person who is responsible.
- In case of immediate interventions to prevent any damage, SBCC and DLC can take suitable measures and the expenses incurred will be recovered from the person responsible.
- All approvals shall be for a period of four years at first instance, renewable for two years at a time.
- GEAC shall have powers to revoke approvals in case of:

  (a) any new information on harmful effects of GMOs.

  (b) GMOs cause such damage to the environment as could not be envisaged when approval was given.

  (c) Non-compliance of any conditions stipulated by GEAC.

4. Guidelines and Manuals

To ascertain the safety of GE derived products several Guidelines have been put in place for ensuring the safety of products derived from GEOs like the following:

4.1. Contained Use

- Recombinant DNA Safety Guidelines, 1990
- Recombinant DNA Safety Guidelines and Regulations, 1994
- Revised Guidelines for Research in Transgenic Plants, 1998
- Regulations and Guidelines for Recombinant DNA Research and Biocontainment, 2017

4.2. Confined Field Trials (CFTs)

- Guidelines for Conduct of Confined Field Trials (CFTs) 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

4.3. Food Safety Assessment

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

4.4. 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

5. Procedure for Development of a Genetically Engineered (GE) Crop in India

The process of development of a Genetically Engineered (GE) crop starts at the Institute level and the following are the different stages at which the applicant needs to obtain approvals from various agencies. The process of approval of GE crops is given in Fig. 1.

a) Any Institute / Organisation/ University which desires of undertaking research and development activities on GE crops needs to constitute IBSC, which reviews and monitors the progress of the project at regular intervals (IBSC meets at least once on a quarterly basis) and it has about 7-8 Members including one DBT nominee.

b) Based on the recommendations of the IBSC, the applicant obtains necessary approvals from RCGM housed in DBT for research till contained/ laboratory conditions. RCGM consists of about 25-30 subject specific experts as its Members.

c) The GE plant is tested for its performance in contained conditions through Event Selection Trials (ESTs) for which applicant needs to obtain approval from RCGM and GEAC. GEAC has about 25-30 subject specialist experts as Members of the Committee.

d) Later the GE Plant is tested in CFTs conditions like Biosafety Research Level (BRL)-I wherein the size of each location would be less than or equal to one acre and the cumulative area not more than 20 acres. These trials are conducted mostly under the supervision of Field Trial In-charge and these experiments are usually done in State Agricultural Universities (SAUs) or ICAR Institutes under their direct supervision. The objective of these trials is to generate adequate plant material for undertaking the above mentioned health and environmental biosafety assessment tests as per relevant Guidelines and Protocols. These trials are recommended by RCGM and approved by GEAC.

e) Based on the results of the BRL-I trials, the applicant is allowed to undertake BRL-II Trials wherein each plot size would not exceed more than 2.5 acres and the number of locations approved for these trials depend on a case to case basis. These trials are recommended and also approved by GEAC.

f) The applicant is required to complete at least three years of BRL trials (BRL-I and BRL-II together) prior to submission of an application for environmental release.

g) During the conduct of each trial, a Central Compliance Committee (CCC) visits each trial at least twice. The Members of the CCC would consist of a Chairman, 1-2 Subject Experts, Representative of RCGM/GEAC, representative of state department of agriculture not less than the rank of Deputy Director (Agriculture), Director of Research of concerned SAU, based on the trait selected, an Entomologist/ Agronomist from the concerned state SAU.

h) Pursuant to completion of the all the above experiments and generation of adequate health and environmental safety data, a detailed dossier consisting of results of all these tests would be submitted to GEAC for its evaluation.

i) GEAC upon evaluation of entire biosafety dossier and after seeking comments from public may recommend the Environmental/ Commercial release of GM crop in India to the Ministry (MoEF&CC).

j) In view of the above mentioned facts, it is submitted that the development of a GE crop takes about 8-10 years of rigorous research, monitoring and evaluation at each stage by several committees consisting of subject matter experts and this development process also involves several Ministries/ agencies at each step.

k) The State Government plays a very critical role in the overall process, as the conduct of each BRL trial is carried out on the receipt of No Objection Certificate (NOC) from the concerned State Department of Agriculture since the year 2010. A representative of State Department of Agriculture, is always one of the member of CCC during the visit of each BRL trial.

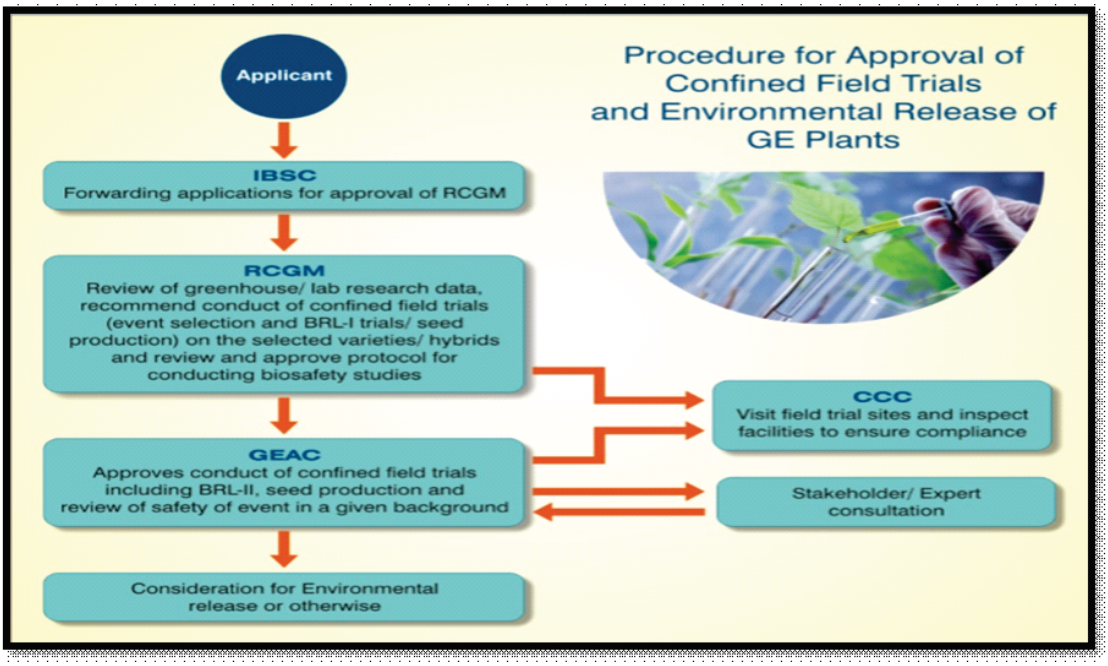


Fig. 1. Process of approval of GE crops in India

6. References

- Department of Biotechnology (DBT) (1990) *Recombinant DNA Safety Guidelines*. http://geacindia.gov.in/guidelines-and-protocols.aspx. Retrieved May 24, 2017.
- Department of Biotechnology (DBT) (1998) *Revised Guidelines for Research in Transgenic Plants*. http://dbtbiosafety.nic.in/. Retrieved May 24, 2017.
- Department of Biotechnology (DBT) (2008) *Protocols for Food and Feed Safety Assessment of GE Crops*. http://dbtbiosafety.nic.in/. Retrieved May 24, 2017.
- DBT and MoEF&CC (2008) *Guidelines and SOPs for Confined Field Trials of Regulated GE plants*. http://dbtbiosafety.nic.in/. Retrieved May 24, 2017.
- Indian Council of Medical Research (2008) *Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants*. http://dbtbiosafety.nic.in/. Retrieved May 24, 2017.
- Ministry of Environment, Forest and Climate Change (MoEF&CC) (1989) G.S.R. 1037 (E); Rules for manufacture, use/import/export & storage of hazardous microorganisms/ genetically engineered organisms or cells. http://geacindia.gov.in/acts-and-rules.aspx. Retrieved May 24, 2017.

- MoEF&CC (2016) Genetically engineered plants in the product development pipeline in India: Results from a survey conducted under the auspices of the phase II Capacity building project on biosafety. http://in.biosafetyclearinghouse.net/phase2/publications.shtml. Retrieved May 24, 2017.

- MoEF&CC and Biotech Consortium India Limited (2015) *Frequently Asked Questions about GE Plants,* Phase II Capacity building Project on Biosafety. http://in.biosafetyclearinghouse.net/phase2/publications.shtml. Retrieved May 24, 2017.

- MoEF&CC and Biotech Consortium India Limited (2015) *Regulatory Framework for GE Plants in India*, Phase II Capacity Building Project on Biosafety. http://in.biosafetyclearinghouse.net/phase2/publications.shtml. Retrieved May 24, 2017.

- MoEF&CC and DBT (2016) *Environmental Risk Assessment of GE Plants; Guidelines, User's Guide and Risk Analysis Framework*. http://geacindia.gov.in/guidelines-and-protocols.aspx. Retrieved May 24, 2017.

Role of Customs Officials in the Implementation of Cartagena Protocol on Biosafety

5

Chimata Murali Krishna

Ministry of Environment, Forest and Climate Change, Govt. of India
New Delhi 110003, India
Email: *cm.krishna@gov.in*

1. Introduction

The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity (CBD) is an international treaty governing the movements of living modified organisms (LMOs) resulting from modern biotechnology from one country to another. It was adopted on 29 January 2000 as a supplementary agreement to the CBD and entered into force on 11 September 2003. As on date, 171 countries are Parties to the Protocol meaning that they have agreed to be bound by its terms. The Protocol was negotiated in the context of many countries not having regulatory systems in place to govern the introduction of LMOs. These countries were concerned that new organisms could be imported into their territories and introduced into the environment without their prior approval or without them even being aware that this was taking place. Many countries were also concerned about the possible impacts LMOs could have on the environment. These concerns included the potential for LMOs to become pests, to out-compete and replace wild relatives, to increase dependence on pesticides or to spread their introduced genes to weedy relatives, potentially creating 'super-weeds'. Countries thus sought an international treaty that would assist them in taking decisions on LMOs. The result was the CPB.

The objective of the Protocol is to, in accordance with the precautionary approach, contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of LMOs resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.

A LMO is defined in the CPB as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. In everyday usage,

LMOs are usually considered to be the same as genetically modified organisms (GMOs), but definitions and interpretations of the term GMO vary widely. The Protocol is an environmental agreement so it uses the term LMOs as these are the organisms that may enter the environment and impact biodiversity. In general, an LMO is made by taking a gene (a piece of DNA) from one organism and inserting it into the DNA of another organism. Scientists search for genes that correspond to desired characteristics. By inserting these genes into other organisms, scientists can create organisms that display the traits coded for by the gene.

Most of the LMOs that have been developed to date are agricultural crops that have genes inserted which make them resistant to certain insects or tolerant of different herbicides. Examples of modified crops include maize, soybean, cotton and canola. These agricultural crops are currently the most widely traded LMOs and so are the LMOs that customs and border control officers are most likely to encounter in their work. Other types of GMOs that are being developed include salmon modified to grow more quickly and mosquitoes modified to reduce the incidence of dengue fever.

The Protocol establishes various rules and procedures for regulating the transboundary movement of LMOs. These are intended to ensure that LMOs do not adversely affect biological diversity and human health. The Protocol aims to ensure the safety of LMOs, not to prohibit their trade. The Protocol also establishes an informational exchange system known as the "Biosafety Clearing-House" (BCH). Parties to the Protocol are required to share certain types of information and decisions via the BCH. The BCH will also be useful to the work of customs and border control officers in their roles in implementing the Protocol.

2. Role of Customs and Border Control Officers in the Implementation of the Protocol

Trade in environmentally sensitive products such as LMOs is a growing global challenge. There is a need for international cooperation to monitor and control the cross-border movement of such products in order to protect the environment and human health. Customs and border control officers have a crucial role to play in addressing the challenge.

A country importing LMOs may wish to ensure that it has approved the LMOs contained in a shipment for their intended use in order to fulfill the objective of the Protocol and to do this, the following to be done:

- The documentation that accompanies a shipment that contains LMOs must identify the shipment as such.
- The sampling of shipments and the detection of any LMOs contained therein can be

used to verify the documentation.

- The documentation and detection of LMOs in a shipment can be used to check whether the competent national authority has approved the LMOs for their intended use in the country.

2.1. Customs and Border Control Officers have the following Roles to Play under the Protocol

- Verifying that the necessary identification information has been provided in the accompanying documentation.
- Inspecting incoming shipments of LMOs.
- Verifying that LMOs for import have received necessary approvals.
- Detecting unintentional or illegal transboundary movements.

The Protocol sets requirements for information that must be included in documentation that accompanies transboundary movements of LMOs. These requirements can be found in Article 18 of the Protocol as well as associated decisions of the Conference of the Parties serving as the meeting of the Parties to the Protocol (the governing body of the Protocol) The information requirements vary depending on the intended use of the LMO. The Protocol distinguishes between different intended uses of LMOs because the different uses pose different risks for biodiversity.

3. Documentation Accompanying LMOs for Intentional Introduction into the Environment must

- clearly identify the content as LMOs and briefly describe the organisms (e.g. the name and relevant traits or characteristics of the organism, its unique identifier).
- specify any requirements for the safe handling, storage, transport and use.
- list the name and address of the importer and exporter - provide an emergency contact point.
- contain a declaration that the movement is in conformity with the requirements of the Protocol applicable to the exporter.

- provide further information, where appropriate, such as the commercial name, risk class and import approval for the LMO.

4. Documentation Accompanying LMOs for Direct Use as Food or Feed, or for Processing (LMOs-FFP) must Clearly State

- that the shipment "contains LMOs-FFP" where the identity of the LMOs is known.
- that the shipment "may contain one or more LMOs-FFP" where the identity of the LMOs is not known.
- that the LMOs are not intended for introduction into the environment.
- the common, scientific and commercial names of the LMOs.
- the transformation event code or its unique identifier (where available).
- the internet address of the BCH for further information.

5. Documentation Accompanying LMOs for Contained Use must

- clearly identify the content as LMOs and indicate that they are "destined for contained use".
- list the name and address of the consignee, exporter and importer.
- specify any requirements for the safe handling, storage, transport and use.
- provide further information, where appropriate, such as the commercial name of the LMOs, the new or modified traits, the transformation event, risk class, use and any unique identification code.

6. Key Roles and Responsibilities of Customs Officers

- The foremost requirement is to ensure that the shipments of LMOs are accompanied by appropriate identification documentation (as mentioned in Documentation chapter).

  Inspecting the documentation accompanying the incoming shipments to verify that the necessary identification information has been provided, the actual content and cross-check them against the accompanying documentation that the documentation corresponds to the actual LMOs in the shipment. It is to be ensured that the documentation is complete and meets the applicable identification requirements specified in the Protocol and the

domestic laws. They may also enforce if any special handling/packaging is required.

- Ensuring that LMOs for import have received necessary approvals.
- Detecting unintentional or illegal transboundary movements; and
- Reporting to relevant authorities information concerning shipments of LMOs arriving at the ports of entry. For this, they should be aware of the rules regarding illegal transboundary movement, country's contact point for notification of potential unintentional transboundary movements, detecting and alerting relevant authorities about illegal or unintentional import or export of LMOs.
- The customs officers will not find the above information in a stand-alone document accompanying shipments of LMOs. Instead, the information that is to be provided will be included in existing types of shipping documentation such as invoices, bills of lading, way bills, etc.

7. Custom Officials Need to be Familiar with

- Any additional documentation and identification requirements in the domestic regulatory framework.
- How to access information, use available resources, such as the BCH and maintain close contact with the National Focal Point for the Protocol and the National Competent Authorities.

8. Inspecting Incoming Shipment of LMOs

- When a shipment of LMOs arrives at customs control point, customs officers need to follow their country's rules and procedures regarding inspection of the shipment to verify its content and cross-check against the accompanying documentation. As it is not possible to visually distinguish a LMO from a conventional organism so verifying the content of a shipment will require (by Plant Quarantine Officer) collecting a sample from the shipment and testing it to determine what, if any, LMOs it contains.
- The Protocol does not set specific requirements for methods for the sampling of shipments and detection of LMOs. Countries will need to set their own rules and procedures regarding how to collect a sample from a shipment and what testing procedures to follow to determine whether a sample contains LMOs and if so, which LMOs and in what quantities (by Plant Quarantine Officer).

- Customs officers will likely need to cooperate with other government agencies involved in this type of work at the border, e.g. health or phytosanitary/ plant quarantine inspectors and associated laboratories.

- Customs officers also need to ensure that shipment of LMOs are handled, stored and packaged according to any applicable requirements specified in the shipping documentation.

- Verifying that LMOs for import have received the necessary approvals either on the basis of the LMOs identified in the shipping documentation or on the basis of the LMOs identified through testing. Customs officers can use the BCH to verify whether these organisms have received the necessary approvals for import into their country.

- Use unique identifiers as a simple way to search the BCH to find information and countries' decisions on the LMO.

9. Detecting Unintentional or Illegal Transboundary Movements

To help prevent unintentional transboundary movements, customs officers need to follow the requirements for the handling, storage, transport and use of the LMOs that should be indicated in the shipping documentation. Unintentional transboundary movements could occur through such means as gene flow as part of natural plant reproduction processes, or accidental contamination due to a spill while a shipment is in transit. If a spill occurs or an unintentional transboundary movement is detected, then contact the country's competent national authority under the Protocol. The Protocol also addresses unintentional and illegal transboundary movements.

The Protocol defines an illegal transboundary movement of an LMO as the one that is carried out in contravention of domestic measures (Article 25). Customs officers will need to be familiar with their national biosafety laws in order to know what constitutes an illegal transboundary movement. If an illegal transboundary movement is detected, it should be immediately informed to the country's competent national authority.

Parties to the Protocol have an obligation to make available to the BCH information concerning cases of illegal transboundary movements pertaining to them.

10. Customs Officers and Biosafety Clearing-House

Biosafety Clearing-House (BCH) is a mechanism set up by the CPB to facilitate the exchange of information on LMOs and assist the Parties to implement their obligations under the

Protocol. Most common types of information that customs officers need to find in the BCH are: Contact information for national authorities, national decisions on whether or not the import of specific LMOs is allowed i.e. LMOs have been approved for import; to verify if the shipment has proper import approvals, check the decisions posted on the BCH regarding LMOs-FFP approved for domestic use/ marketing etc.

They also need to be familiar with the Advance Informed Agreement (AIA) procedure and the domestic decision-making procedures and requirements, which LMOs our country has subjected to a simplified procedure or exempted from the AIA procedure and any bilateral or multilateral agreements/arrangements.

BCH has also created a Collaborative Portal for Customs officials to facilitate implementation of the protocol and strengthen the capacities of the enforcement agencies.

11. Information on LMOs in Different Countries

Globally, 11 crops are commercialized in 24 countries (ISAAA, 2017). The GM crops being grown in different countries are given in Table 1 and the list of GM crops and traits approved in different countries is given in Table 2. The list of GM crops and events approved in different countries is given in Chapter 2. This information is of paramount importance to the customs officials.

Table 1. Country-wise commercialized GM crops

S. No.	Country	GM crop	S. No.	Country	GM crop
1.	Argentina	Cotton, maize, soybean	7.	Mexico	Cotton
2.	Australia	Canola, cotton	8.	Myanmar	Cotton
3.	Bangladesh	Brinjal/eggplant	9.	Pakistan	Cotton
4.	Bolivia	Soybean	10.	Paraguay	Cotton, maize, soybean
5.	Brazil	Cotton, maize, soybean	11.	Philippines	Maize
6.	Canada	Alfaalfa, canola, maize, soybean, sugar beet, potato	12.	Portugal	Maize

13.	Chile	Canola, maize, soybean	19.	South Africa	Cotton, maize, soybean
14.	China	Cotton, papaya	20.	Spain	Maize
15.	Colombia	Cotton, maize	21.	Sudan	Cotton
16.	Costa Rica	Cotton, Pine apple	22.	Uruguay	Maize, soybean
17.	Honduras	Maize	23.	USA	Alfalfa, apple, canola, cotton, maize, papaya, potato, soybean, squash, sugar beet
18.	India	Cotton	24.	Vietnam	Maize

Table 2. List of GM crops and traits approved in different countries

S. No.	GE plants	Traits/uses	Countries where approved
1.	Alfaalfa	Herbicide tolerance	USA
2.	Apple	Anti-brushing and anti-browning	USA
3.	Canola	Herbicide tolerance and improved protection against weeds	Canada, USA, Australia, Chile
4.	Carnation	Modified flower colour and herbicide tolerance	Australia, Columbia
5.	Cotton	Improved insect protection, herbicide tolerance and improved protection against weeds	Australia, USA, China, Mexico, South Africa, Argentina, India, Columbia, Burkino Fasco, Sudan, Pakistan, Brazil, Myanmar, Paraguay, Costa Rica
6.	Egg plant (Brinjal)	Insect resistance	Bangladesh
7.	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
8.	Papaya	Virus resistance	USA, China
9.	Petunia	Modified flower colour	China
10.	Poplar	Insect resistance	China
11.	Potato	Improved quality, anti-bruising and anti-browning	USA
12.	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
13.	Squash	Resistance against *Watermelon mosaic virus* and *Zucchini yellow mosaic virus*	USA
14.	Sugar beet	Herbicide tolerance	USA, Canada
15.	Sweet pepper	Virus resistance	China
16.	Tomato	Delayed ripening and virus resistance	China

Source: ISAAA (2016)

12. References

- http://bch.cbd.int/help/trainingmaterials/En/03)%20Training%20Modules/MO11En.pdf a. Retrieved October 10, 2014
- http://nacen.gov.in/inspire/uploads/downloads/53c78bac556c1.pdf. Retrieved June 2,2015.
- https://bch.cbd.int/onlineconferences/portal_art18/htpi_customs_materials.shtml Retrieved October 12, 2013.
- https://www.google.co.in/#q=biosafety+clearing+house+custom+official+portal Retrieved November 15, 2014.
- https://www.yumpu.com/en/document/view/43261159/the-role-of-customs-officials-and-biosafety-clearing-house. Retrieved October 10, 2014.
- The Cartagena Protocol on Biosafety. http://www.biodiv.org/biosafety. Retrieved October 25, 2008.
- ISAAA (2016) *Global Status of Commercialized Biotech/GM Crops* . ISAAA Brief No. 52. ISAAA: Ithaca, NY. Available at: http://www.isaaa.org/. Retrieved June 10, 2018.
- http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S1727-37812014000500002.
- ISAAA (2017) *Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years* . ISAAA Brie*f* No. 53. ISAAA: Ithaca, NY. http://www.isaaa.org/. Retrieved June 28, 2018.

National Plant Quarantine System

6

SC Dubey, V Celia Chalam and Shashi Bhalla
ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
Email: *sunil.dubey@icar.gov.in*

1. Introduction

Introduction of useful planting material from other countries has played a significant role in diversifying Indian agriculture and boosting the production. However, introduction of seed and planting material without proper inspection for associated pests may prove disastrous as evident from several examples of pest epidemics in the past. Therefore, plant quarantine assumes special importance in safe exchange of plant material. Plant quarantine is a government endeavour enforced through legislative measures to regulate the introduction of planting material, plant products, soil, living organisms etc. in order to prevent inadvertent introduction of insect pests, pathogens and weeds harmful to the agriculture of a country/ state/region and if introduced, prevent their establishment and further spread. A quarantine pest is, the pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed, and being officially controlled (https://www.ippc.int/largefiles/adopted.../en/ISPM_05_2007_En_2007-07-26.pdf).

The devastating effects resulting from pests introduced along with international movement of seeds and other planting material are well documented (Khetarpal *et al.,* 2006). The Irish famine of 1845, which forced the people to migrate *en masse* from Europe, was the result of almost total failure of potato crop due to attack of late blight pathogen *(Phytophthora infestans)* introduced from Central America. Coffee rust (*Hemileia vastatrix)* appeared in Sri Lanka in 1875 and reduced the coffee production by >90% in 1889. The disease entered India in 1876 from Sri Lanka and within a decade, the coffee industry of South India was badly affected. Like in other countries, a number of exotic plant pests got introduced into India along with imported planting material causing serious crop losses from time to time. These introductions highlight the fact that increased international travel and trade, had exposed the country to the danger of infiltration of exotic pests harmful to our agriculture.

2. Plant Quarantine Regulatory Framework in India

The Directorate of Plant Protection, Quarantine and Storage (DPPQS) was created in 1946,

under the Ministry of Agriculture (now Ministry of Agriculture and Farmers' Welfare (MoA&FW), with Plant Protection Adviser to the Govt. of India, as its Head. Earlier the customs department implemented the plant quarantine regulations. Three categories of materials are being imported under the Plant Quarantine (Regulation of Import into India) Order, 2003 (herein after referred as PQ Order): (a) bulk consignments for consumption, (b) bulk consignments of seeds/planting material for sowing/planting, and (c) samples of germplasm in small quantities for research purposes. The Plant Quarantine Stations under the DPPQS undertake quarantine processing and clearance of consignments of the first two categories located in different parts of the country. At present there are 53 Plant Quarantine Stations operational at major airports, seaports and land frontiers. However, the PQ Order has identified >130 points of entry for import of seeds, plants, plant products and other articles. It has specified plants/ planting material that are (i) prohibited to import into India (ii) permitted for import with additional declarations, and (iii) permitted to import under restricted conditions. Import permit (IP) and Phytosanitary Certificate (PC) are essential for importing plant material into the country.

There are 41 Inspection Authorities who inspect the consignment being grown in isolation in different parts of the country for presence of exotic pests. Besides, the DPPQS has developed 22 national standards on various phytosanitary issues such as on pest risk analysis, pest-free areas for fruit flies and stone weevils, certification of facilities for treatment of wood packaging material, methyl bromide fumigation etc. Also, six Standard Operating Procedures have been notified including Export inspection and phytosanitary certification of plants/ plant products and other regulated articles, post-entry quarantine inspection etc. Some important pests were detected and intercepted in planting material imported into India for commercial purposes (http://plantquarantineindia.nic.in/PQISMain/Default.aspx).

Indian Council of Agricultural Research- National Bureau Plant Genetic Resources (ICAR-NBPGR), a nodal organisation for management of plant genetic resources in India is vested with the authority to issue Import Permit and Phytosanitary Certificate and undertake quarantine processing of all seed material and plant propagules of germplasm including transgenic planting material exchanged for research purposes. ICAR-NBPGR has a Plant Quarantine Regional Station at Hyderabad to undertake quarantine of germplasm of mandated crops of ICRISAT and also of public and private sector organisations in southern India. However, quarantine processing of transgenics germplasm is undertaken at New Delhi only.

All the imports are made as per the provisions of the PQ Order. The documents, which should essentially accompany the consignment, include Import Permit issued from the country of import as per PQ 09 of PQ Order 2003 giving details of material with additional declaration

that the material is free from the specific pests (as per Schedules V and VI of the PQ Order 2003) and Phytosanitary Certificate issued by the country of export giving details of material and treatment as per the International Plant Protection Convention (IPPC) format. The Schedule IV includes 14 crops and countries from where import is prohibited along with the name of pest(s). The Schedule V includes 17 crops with restricted import permissible only with the recommendation of authorized institutions with additional declarations and special conditions. The Schedule VI includes 693 crops permitted to be imported with additional declarations required to be incorporated into Phytosanitary Certificate and special conditions. A total of 1235 pests are included as regulated/quarantine pests for India in the PQ Order 2003 (Bhalla *et al.*, 2018).

3. PQ Order 2003: Provisions for Import of Living Modified Organisms (LMOs)/ Genetically Modified Organisms (GMOs)/ Transgenics

The germplasm of living modified organisms (LMOs)/genetically modified organisms (GMOs) /transgenic material with desirable traits is being imported into the country for various research programmes. Presently, in India, import of transgenic planting material is permitted only for research purposes as per the PQ Order. ICAR-NBPGR is the nodal agency to issue Import Permit, Phytosanitary Certificate and undertake quarantine of germplasm including transgenics under exchange.

4. General Conditions/ Procedure for Import of Transgenic Planting Material for Research

ICAR-NBPGR has brought out the Guidelines for import and quarantine of transgenic Planting Material (Anonymous, 2017).To import transgenic planting material for research purposes, the indenter (public/ private organisations) should follow the following:

4.1. Apply to RCGM for technical clearance through IBSC in the prescribed form. Recently, RCGM has brought out the Simplified Procedures/ Guidelines on Exchange, Import & Export of Genetically Engineered Organisms and Products thereof for Research Purpose.

4.2. Detailed information on the gene/ gene construct/ source of gene and event expression information in the transgenic event along with identity of the event.

4.3. The RCGM issues technical clearance to import the material and the indentor is required to meet all the requirements from the safety point as given below:

- No transgenic material is permitted for experimentation in open environment without prior authorization from Government of India.

- For propagation of transgenic seed in an open environment, a separate application has to be made to RCGM through its IBSC.
- All precautions should be taken to prevent the escape of genetic material into open environment and the Recombinant DNA Safety Guidelines of the Government of India, needs to be followed.
- Full account of the transgenic plants raised from the imported seed planting material is to be kept in a bound book, which should be available for inspection by the authority in case such a need arises.
- All transgenic material preserved by the indenters should be available for inspection, whenever required.
- All the unwanted transgenic material should be destroyed after the experiments have been conducted.
- The transgenic seeds for research purpose would be allowed for import only through ICAR-NBPGR, New Delhi. The applicant needs to certify to ICAR-NBPGR that the material being imported conforms to the description given in the import clearance letter/issued by RCGM.
- ICAR-NBPGR shall retain 5% of the seed, if required by RCGM.
- The exporter of transgenic material shall certify that the transgenic seeds have the genes as described in the permission.
- The exporter shall also certify that the transgenic material do not contain any embryogenesis deactivator gene.

4.4. After obtaining technical clearance from RCGM, apply for Import Permit (IP) to the Director, ICAR-NBPGR, New Delhi in form PQ 08.

- No consignment of transgenics shall be imported into India for research/ experimental purpose without valid permit issued by the Director, ICAR-NBPGR, New Delhi.

4.5. Handling and packing instructions

- The transgenic seeds shall be packed in a durable container of metal or plastic.
- The primary package container shall have a label describing the contents, quantity, traits, date of packing and the safety instructions, if any.

- The primary packing shall be packed in a secondary packing material of durable plastic or any other material to ensure safe handling of the transgenic material.
- The package shall have a packing insert that describes the name, quantity, traits, date of packing, handling and storage instructions, safety precautions, if any.
- The transgenic traits shall state the name of the gene, the marker if any and any other genetic material and the purpose for which the transformation has been carried out.
- The packing insert shall be in the font size that is easily readable by the recipient and the authorities who would be inspecting the material before release.

4.6. For issue of IP, a fee of Rs.350/- for public sector and Rs.700/- for private sector excluding GST @ 18% shall be payable along with the application for the import of transgenics and the fee shall be payable in the form of Demand Draft payable to the Director, ICAR-NBPGR, New Delhi (http//www.nbpgr.ernet.in).

4.7. The IP issued shall be valid for a period of six months from the date of issue. The Director, ICAR-NBPGR, may, on request, extend the period of validity for a further period of six months after charging Rs.75/- for public sector and Rs.100/- for private sector as a revalidation fee provided such a request for extension of validity is made before the expiry of the permit with adequate reasons to be recorded in writing.

4.8. The Director, ICAR-NBPGR issues IP in form PQ 09 (Appendix III) in triplicate, if satisfied that the applicant meets all the necessary conditions.

4.9. One copy of IP shall be forwarded to the exporter in advance to facilitate incorporation of IP number in the Phytosanitary Certificate (PC) issued by the authorized officer at the country of origin with the additional declaration that the material is free from pests mentioned under Schedule V and VI of PQ Order or that the pests as specified do not occur in the country or state of origin as supported by documentary evidence thereof.

4.10. A red/ white tag in form PQ 11 shall be issued.

4.11. The IP issued shall not be transferable and no amendments to the permit shall be issued.

4.12. No consignment of seed or grain shall be permitted to be imported with contamination of quarantine weeds, which are listed in Schedule VIII unless the said consignment has been devitalized by the exporting country and a certificate to that effect has been endorsed in the PC issued by the exporting country.

4.13. No consignment shall be permitted for import unless accompanied by an original copy of the PC issued by an authorized officer at the country of origin in the form PQ 21.

4.14. Handling and cargo clearance charges for consignments received at the Airport (Rs.2,000/- for public sector; Rs.4,000/- for private sector).

- All imports of germplasm including transgenic planting material shall be permitted only through New Delhi Airport.
- No imported consignments of germplasm including transgenics shall be opened at the point of entry and it shall be forwarded directly to the Director, ICAR-NBPGR, New Delhi.

4.15. Quarantine processing fee for transgenics is given below:

- Seed: For each seed sample, Rs.750/- for public sector and Rs. 1,500/- for private sector.
- Vegetative Propagules (VP) / Tissue Culture (TC) Tubes:
- One sample up to 10 Vegetative Propagules (VP)/ 10 Tissue Culture (TC) tubes- Rs.375/- for public sector and Rs.750/- for private sector.
- For every additional VP/ TC tube in a sample (11-100 VP/ TC tubes maximum- Rs.30/- for public sector and Rs.75/- for private sector.
- GST @ 18% as per prevailing Government orders shall be applicable.

4.16. Besides the above requirements, post-entry quarantine inspection (PEQI) of all imported transgenic material grown by the indenter is required to be done by the scientist(s) from ICAR-NBPGR to ensure its freedom from exotic pests.

5. Methodology for Quarantine Processing of Transgenics

5.1. Verification of Documents

After receiving the consignment, it is ensured that it is accompanied with the following documents:

- Import Clearance Letter from RCGM (This authorization was vested upon RCGM vide Govt. of India Notification No. GSR 1037(E) dated 05.12.1989).
- Import Permit issued by ICAR-NBPGR, New Delhi (This authorization was vested upon ICAR-NBPGR vide Govt. of India Notification No. GSR 1067(E) dated 05.12.1989).

- Phytosanitary Certificate from the country of export.
- Supplier's certificate that the material contains the declared genes for which the technical clearance from RCGM has been accorded and does not contain embryogenesis deactivator genes.

5.2. Check-list of Pests Associated with Import of Transgenics from the Source Country

The pest-risk associated with the import of a transgenic material from a particular country is analysed by preparing checklist of pests associated with seeds/planting material of different crops. Information is collected from the available literature on geographical distribution and epidemiological parameters in order to assess the level of risk prior to import, so as to facilitate their release after quarantine processing in a more efficient and effective manner.

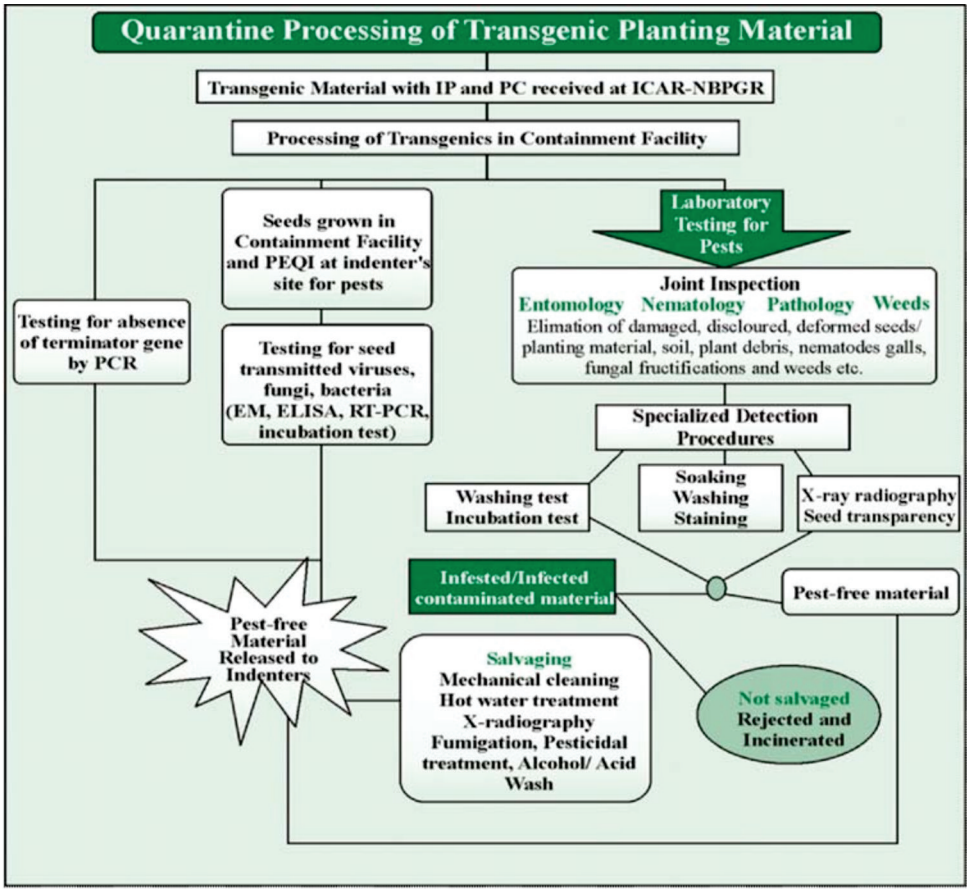
5.3. Methodology for Detection and Identification of Pests

There are *State of art* facilities at ICAR-NBPGR to undertake quarantine activities and to disinfest/ disinfect the germplasm so as to release it in a pest-free state. ICAR-NBPGR has established a Containment facility (CL-4), for quarantine of imported transgenic planting material and post-entry quarantine growing of the transgenic planting material. Number of techniques have been developed/ standardized over the years for the detection of various pests.

A methodology for simultaneous testing of transgenics for detection of associated pests (insects, mites, nematodes, fungi, bacteria, viruses and weeds) and transgenes including embryogenesis deactivator gene has been developed for the purpose (Fig. 1).

The different methods followed for detection and identification of various pests are described. However, the methodology is adopted according to the pest species.

5.3.1. Visual Inspection: All the samples are first examined visually. It is done either by naked eye or with the help of magnifying glass/ stereobinocular microscope for detecting the presence of dead or actively moving larval and adult stages of insects, damaged or deformed or discoloured seeds, plant parts, contaminants, dust, flour, webbing, presence of excreta, soil clods, galls and swellings on roots, lesions, fungal sclerotia, rust spores on seed surface, smut balls, fungal frucitifications and weeds etc.



ELISA: Enzyme-linked Immunosorbent Assay, EM: Electron Microscopy, IP: Import Permit; PC: Phytosanitary Certificate, PEQI: Post-entry Quarantine Inspection; RT-PCR: Reverse Transcription-Polymerase Chain Reaction

Fig. 1: Flow chart for quarantine processing of imported transgenic planting material

5.3.2. Specialized Tests: These are used for detection of different groups of pests and are given below:

5.3.2.1. Insects and Mites

X-ray radiography, used to detect seeds infested with phytophagous chalcidoids, bruchids and certain other insect groups that do not exhibit any external symptoms of damage on seed surface. Based on literature survey and past experience a list of >340 plant genera has been drawn up that are compulsorily subjected to X-ray screening (Bhalla *et al.*, 2003). The soft

X-rays generated at 3mA current and specific kV, distance from source and exposure time, after passing through the seeds produce a distinct image of the healthy and insect infested seeds without affecting the viability and vigour of the seeds. On developing the X-ray plates, insect infested seeds, if present, are hand-picked and healthy seeds are released to the indenter. Of late, with the use of real-time X- ray radiography machine the radiograph can be viewed directly on the monitor thus saving time and resources on developing the X-ray plate.

Transparency method is used for detecting infestation in small seeds especially those of family Graminae and Umbelliferae. The seeds are kept in lacto-phenol solution (phenol, lactic acid, distilled water and glycerin in the ratio of 2:2:2:1, respectively) in boiling water in a water bath for 1-2 hours for making these transparent to reveal insect infestation.

5.3.2.2. Plant Parasitic Nematodes

Soaking of seeds known/ suspected to carry seed-borne nematodes in water for overnight softens the seeds, which are teased/ crushed enabling the nematodes, if present, to come out in water.

Soaking of some plant material in water and then sieving through nematological sieves (the finest sieve is of 400 mesh per linear inch) reveals nematodes that are retained on the sieve. These are recovered and examined under the compound microscope for identification.

Staining technique is used for quick detection of nematodes in vegetative propagules where a part of the plant tissue (especially roots) is boiled in acid fuchsin lactophenol solution for a few minutes and de-stained in clear lactophenol. The nematodes, if present, retain the red stain more deeply than the plant tissue and can easily be detected under stereo microscope.

Examination of accompanying soil shows the presence of viable nematodes especially ectoparasites and cysts of cyst forming nematodes.

5.3.2.3. Fungal and Bacterial Pathogens

All the samples are first examined visually and then under stereo-binocular microscope for the presence of fungal mycelium/ fructifications such as ergot sclerotia, rust pustules, smut and bunt balls and for symptoms such as discolouration, deformation, malformation etc.

Seeds suspected to be contaminated with rust/ smut/ mildews are subjected to washing test by adding a small amount of distilled water in a test tube. The tube is shaken on a mechanical shaker for two minutes and the supernatant examined under the stereo-binocular microscope and compound microscope for detection of oospores/spores.

Blotter method is used for detection of fungal and bacterial pathogens capable of producing mycelial growth and fruiting structures after the incubation. Seeds are placed on 3-4 layers of moist filter paper in plastic Petri plates and incubated at 20±1°C under fluorescent tubes in alternating cycles of 12 hours light/ darkness for 7 days. Observations are recorded on 8th day under stereo-binocular microscope for growth of associated fungi and bacteria. Slides are also prepared and observed under compound microscope, wherever needed.

Agar culture-method-seeds after surface sterilization are placed on culture media in Petri plates and incubated as in blotter method and examined for growth of associated fungi/ bacteria. The pathogens are isolated, purified and tested for colony characters, morphological and cultural characters etc.

NaOH seed soak method - seeds are soaked in 0.2% NaOH solution for 24 hrs at 20°C. When examined infected seeds show shiny jet black discoloration which upon rupture releases a stream of spores.

5.3.2.4. Plant Viruses

The seeds showing virus-like symptoms are removed and healthy-looking seeds are subjected to grow-out test.

Post-entry Quarantine Growing in Containment Facility and at Indenter's Site: ~Five seeds each from a sample are grown in the Containment Facility at ICAR-NBPGR, New Delhi for about 45 days for detection of seed-transmitted pathogens which are not detectable in the laboratory tests. On the basis of observations at regular intervals, suspected leaf/ soil samples are tested in the laboratory for the presence of various pests. Thereafter, the plants are uprooted and disposed off as per biosafety guidelines in the presence of Institutional Biosafety Committee (IBSC) members.

Crops grown at indenter's site in contained conditions are also inspected and seedlings/ plant parts suspected to be infected are processed in laboratory for detection, confirmation of the associated pests. Plants infected by pests of quarantine significance of India are uprooted and incinerated.

The seedlings showing virus-like symptoms and representative healthy-looking samples are further subjected to different techniques viz., infectivity test/ electron microscopy/ enzyme-linked immunosorbent assay (ELISA)/ reverse transcription-Polymerase chain reaction (RT-PCR) etc. A number of biological, physical, serological and molecular techniques are available for virus detection. These techniques are adopted both alone or in combination based on their availability (Chalam and Khetarpal, 2008) and need, and are given below:

Infectivity test is done to assay the presence of virus by inoculating leaf extracts of seedlings showing symptoms on indicator hosts. This method reveals the symptom-less or latent infections of plants not observed in grow-out tests.

Observation of sample from leaf showing viral symptoms under the transmission electron microscope reveals the size and shape of the virus particles, if present.

For serological diagnosis of plant viruses, ELISA, a relatively simple, rapid and sensitive technique is used for simultaneous testing of a large number of samples.

For detection of low concentration of viruses and/ or for confirmation of doubtful ELISA results, the technique of RT-PCR is adopted for detecting RNA viruses.

Based on the interception of the pest, the treatment is given to the samples.

Since 1997, ICAR-NBPGR has imported >15,000 accessions of 15 crops (*Arabidopsis thaliana, Brassica juncea, B. napus, B. oleracea, Cicer arietinum, Eucalyptus, Glycine max, Gossypium hirsutum, Manihot esculenta, Nicotiana tabacum, Oryza sativa, Solanum lycopersicum, S. tuberosum, Triticum aestivum* and *Zea mays*) and exported 41 samples of *O. sativa* and 19 of *A. thaliana*. Over the years, during quarantine processing, a number of pests of quarantine significance have been intercepted in transgenics germplasm. The important interceptions include *Peronospora manshurica* on soybean; *Barley stripe mosaic virus* and *Wheat streak mosaic virus* on corn and wheat, *Bean mild mosaic virus, Cherry leaf roll virus, Cowpea mottle virus, Cowpea severe mosaic virus, Raspberry ringspot virus, Tomato ringspot virus* on soybean, High plains virus and *Maize chlorotic mottle virus* on corn (Singh *et al*., 2003; Bhalla *et al*., 2008; Chalam *et al.*, 2017).

5.4. Disinfection/ Disinfestation of Infected/ Infested/ Contaminated Material

Infected/ infested/ contaminated material is salvaged by various disinfection/ disinfestation treatments/ techniques. The following are the various methods used for salvaging:

(a) The soil, plant debris, weeds, discoloured, deformed and shriveled seeds are mechanically cleaned by hand-picking. The vegetative propagules are cleaned by excising the infected portion.

(b) Hot water treatment (HWT) with various temperature and time combinations is used for eliminating pathogens like fungi, bacteria, insects and nematodes. The treatment is given in HWT tank fitted with heaters of different capacities, stirrer, thermostat and/ or contact thermometer for controlling the water temperature.

(c) X-ray radiography is used to separate insect infested seeds (which do not have any external symptoms) from healthy ones. On developing the film exposed to soft X-rays, the infested seeds can be easily distinguished and are hand-picked from the seed geometry. X-ray radiography is used both for detection as well as salvaging of the infested material.

(d) Fumigation is one of the most effective methods used in quarantine for eliminating insects, and is done either at atmospheric pressure or under vacuum conditions. Atmospheric fumigation is done at normal air pressure in an air tight container using suitable fumigant at recommended dose and duration - Ethylene dichloride carbon tetrachloride (EDCT) mixture (3: 1) at 320 mg/l at 30°C for 48 h or with Aluminium phosphide 2 g/cu m at NAP for 72 h. Vacuum fumigation is done in especially designed fumigation chamber which helps in hastening the penetration of the fumigant through tightly packed material for internal infestation.

(e) Pesticidal treatment is the most practical method to use in quarantine for effective control of surface feeding insects, mites and nematodes, etc. Few nematodes in rooted plants, cuttings, tubers and other vegetatively propagated plant material, dipping in systemic chemicals at various concentration for different durations has been found effective. Chemical seed dressing is generally given for eliminating seed-borne fungi and bacteria. Various systemic pesticides are available for use as seed dressing or dip treatment for vegetative propagules.

6. Molecular Testing for Detection of Transgenes

All the imported transgenic lines are tested for specific transgenic elements including transgenes/ markers/ promoters/ terminators along with the taxon-specific endogenous reference gene.

6.1. Detection of Embryogenesis Deactivator Gene

The imported transgenic lines are tested to ensure the absence of embryogenesis deactivator gene. PCR analysis is carried out using the primers specifically designed for *cre* sequence of embryogenesis deactivator gene. Plasmid cloned with *cre* sequence of 1031 bp is used as positive control. In PCR amplification of *cre* sequences amplicon of 1031 bp size amplified only in positive sample while no amplicon of corresponding size has been observed in any of these transgenic samples tested so far.

7. Release of Material to the Indenter

The material free from quarantine pests and terminator gene is released to the indenters with an undertaking (Appendix VI & VII) from the indenter that the material would be grown under contained conditions as per the DBT Guidelines in the supervision of a plant pathologist to report incidence of any seed-borne pathogens.

8. Mechanism for Export of Material

Export of GM plant and planting material attracts the provisions of the Biological Diversity Act (BDA) 2000. As per the provision of Section 3 of BDA, no person from outside India or corporate body, association, organisation incorporated or registered in India having non-Indian participation in its share capital or management can access any biological resources or knowledge associated for research, commercial utilization, bio-prospecting or bioutilization, without proper approval of National Biodiversity Authority (NBA). Bilateral agreements/ collaborative projects, however, are exempted which conform to the policy guidelines issued/ approved by the Central Government.

For export also the applicant has to take the approval from the RCGM and request for germplasm export is addressed to the Director, ICAR-NBPGR, New Delhi for issuance of the Phytosanitary Certificate.

The following persons are required to take the prior approval of NBA for export or collection of biological resources:

- A person who is not a citizen of India, a non-resident citizen of India
- A body corporate, association or organisation not registered in India/ or incorporated or registered in India under any law for the time being in force which has any non-India participation in its share capital or management.

After approval by NBA, material is sent to ICAR-NBPGR for quarantine clearance and issue of Phytosanitary Certificate.

9. Perspectives

- Development of serological and molecular diagnostic protocols for detection and identification of exotic viruses, bacteria, fungi and immature stages of insects and the variability therein. Development of digitized keys for the identification of insect pests of quarantine significance.

- Developing eco-friendly treatments for salvaging of infested/ infected plant material.
- The mechanism of import of transgenic material for research purpose is well in place, However, the mechanism for bulk imports of transgenics needs to be streamlined.
- Plant quarantine stations located at various sea ports, airports and land frontiers need to develop infrastructure and expertise to handle and test the bulk imports of GMOs in the coming years.

10. References

- Anonymous (2017) *Guidelines for Import and Quarantine of Transgenic Planting Material*, ICAR-National Bureau of plant Genetic Resources, New Delhi, India. 40 p +iv.
- Bhalla S, Chalam VC, Singh B, Gupta K and Dubey SC (2018) *Biosecuring Plant Genetic Resources in India: Role of Plant Quarantine.* ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. 216 p +vi.
- Bhalla S, Chalam VC, Singh B, Rajan, Gupta K, Firke P, Kumar N, Jain A, Som R, Randhawa GJ, Kapur ML and Khetarpal RK (2008) Quarantine processing of imported transgenic rice and evaluation of risk in import. *Indian Journal of Plant Genetic Resources* 21: 141-145.
- Bhalla S, Kapur ML, Lal B, Verma BR and Charan Singh (2003) Quarantine risk associated with exchange of plant genera carrying hidden infestation. *Indian Journal of Plant Genetic Resources* 15:160-163.
- Chalam VC, Bhalla S, Singh B and Rajan (eds) (2012) *Potential Quarantine Pests for India: Grain Legumes*, National Bureau of Plant Genetic Resources, New Delhi, India. 324p+xii.
- Chalam VC and Khetarpal RK (2008) A critical appraisal of challenges in exclusion of plant viruses during transboundary movement of seeds. *Indian Journal of Virology* 19: 139-149.
- Chalam VC, Parakh DB and Maurya AK (2017) Role of viral diagnostics in quarantine for plant genetic resources and preparedness. *Indian Journal of Plant Genetic Resources* 30: 271-285.

- Dubey SC and Gupta K (2016) Plant Quarantine system for PGR in India. *Indian Journal of Plant Genetic Resources* 29: 410-413.

- http://www.plantquarantineindia.nic.in. Plant Quarantine (Regulation of Import into India) Order 2003, Ministry of Agriculture, Cooperation and Farmers' Welfare, Govt. of India. Retrieved June 14, 2018

- Khetarpal RK, Arjun Lal, Varaprasad KS, Agarwal PC, Bhalla S, Chalam VC and Gupta K (2006) Quarantine for safe exchange of plant genetic resources. In: *Hundred Years of Plant Genetic Resources Management in India* Singh AK, Kalyani Srinivasan, Sanjeev Saxena and Dhillon BS (eds). National Bureau of Plant Genetic Resources, New Delhi, India. 83-108 pp.

- Singh B, Rajan, Bhalla S, Chalam VC, Pandey BM, Singh SK, Kumar N and Khetarpal RK (2003) Quarantine processing of imported transgenic planting material. *Indian Journal of Agricultural Sciences* 73: 97-100.

Documentations Requirement for Transboundary Movement of Living Modified Organisms

7

Shashi Bhalla, V Celia Chalam, Ruchi Sharma and SC Dubey

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
Email: *shashi.bhalla@icar.gov.in; sbhallamail@gmail.com*

1. Introduction

The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity (CBD) is an international Protocol governing the transboundary movement of living modified organisms (LMOs) resulting from modern biotechnology from one country to another. The objective of Protocol is to ensure an adequate level of protection in the field of safe transfer, handling and use of LMOs to avoid adverse effects on the conservation and sustainable use of biological diversity, also considering the risks to human health. The Protocol specifically focuses on transboundary movement of LMOs for the harmonization of biosafety framework under the CBD. However, it does not cover products derived from LMOs (e.g., oil from GM soybean) and LMOs that are pharmaceuticals for humans (http://www.biodiv.org/biosafety). It promotes biosafety by establishing rules and procedures for the safe transfer, handling, and use of LMOs.

Article 18 of the CPB sets forth rules related to Handling, transport, packaging and identification requirements of LMOs and concerns the measures to be taken to avoid risks during transboundary movement of LMOs. It requires Parties to take measures for the safe handling, packaging and transport of LMOs which are subject to intentional transboundary movement. This applies to all LMOs within the scope of the Protocol viz., LMOs for food, feed or processing (LMO-FFPs), LMOs in transit; LMOs destined for contained use as well as LMOs for intentional release. The purpose is to provide information on the requirements for safe handling to those handling LMOs in the Party of import. The three categories of LMOs have specific documentations requirement. Implementation of CPB in India requires strengthening the capacities of enforcement agencies as plant quarantine and customs officials who are the first line of defence for transboundary movement of LMOs so as to enable them to fulfill the requirements of Article 18: Handling, transport, packaging and identification of LMOs of CPB.

As the LMOs are environmentally sensitive products, their trade is a global concern so there is a need for international cooperation to monitor and control the cross-border movement of such products in order to protect the environment and human health. India is a party to the CPB and has obligation for its implementation. In India, Ministry of Environment, Forest and Climate Change (MoEF &CC) is the nodal Ministry for the implementation of the Protocol.

2. **Article 18 of CPB** has three elements

2.1. Para 1 of Article 18 of the Protocol specifies a general obligation on each party to the Protocol to take necessary measures for the safe handling, packaging and transport of LMOs which are subject to intentional transboundary movement. This obligation extends to all LMOs subject to intentional transboundary movement that are within the scope of the Protocol in accordance with Article 4 – i.e. it includes LMOs in transit, LMOs destined for contained use in the Party of import, and LMO-FFPs; but not the transboundary movement of LMOs which are pharmaceuticals for humans that are addressed by other international agreements or organisations (Article 5).

2.2. Para 2 of Article 18 specifies three different sets of requirements for documentation. Each party is required to take measures for the necessary documentation to accompany the shipment according to the intended use of LMOs divided into three categories viz., LMOs for FFPs, LMOs for intentional release and LMOs for contained use. It sets out what information must be provided in documentation accompanying transboundary movements of LMOs.

The information requirements in the documents accompanying shipment vary according to the intended use of the LMOs in question. This information provides a means to identify and track transboundary movement of LMOs; gives information to the Party of import at the border; and offers a contact point for further information about the consignment in question.

2.3. Para 3 of Article 18 provides for possible future modalities for development of standards for handling, transport, packaging and identification of LMOs by the Conference of Parties serving as meeting of the Parties (COP-MOP) to this Protocol and in consultation with other relevant international bodies.

3. Three categories of LMOs are distinguished for the intentiona l transboundary movements of LMOs, under the Protocol. These are

3.1. LMOs Intended for Direct use as Food or Feed, or for Processing (LMO-FFPs)

This represents a large majority of LMOs, i.e. genetically modified crops, such as soybean,

maize, canola, tomato, cotton, etc. The Protocol does not cover consumer products derived from LMOs, such as corn flakes, flour, starch, seed-oil, tomato paste or ketchup. LMOs intended for direct use as food or feed, or for processing (LMO-FFPs), e.g. agricultural commodities such as corn, soybean etc. but are not intended for use as seeds may be subjected to simplified procedures which includes communicating the decision through BCH.

3.2. LMOs Destined for Contained Use

The contained use being defined in Article 3(b) of the Protocol to include activities in which LMOs are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment e.g. bacteria for laboratory experiments and LMOs in transit are exempt from Advance informed Agreement (AIA) procedures.

3.3. LMOs for Intentional Introduction into the Environment

LMOs for intentional introduction into the environment (e.g. seed, live fish) are subjected to AIA and includes communication and decision making process between the parties. The Protocol specifies that the shipment of LMOs subject of transboundary movement must be accompanied by appropriate documentation. It should also specify the identity of LMOs and contact details of persons responsible for such movement. These procedures and requirements provide importing Parties with the necessary information needed for making informed decisions about whether or not to accept LMOs imports and for handling them in a safe manner.

4. Documentations Requirement for Transboundary Movement of LMOs

The documentations requirement vary according to the intended use of the LMOs. Shipment of LMOs must be accompanied by documents that clearly identify these organisms. The identification information may be incorporated into a commercial in-voice or other documents used by existing systems or required by domestic laws.

4.1. Article 18 (2a) Documentations Requirement for LMOs Intended for Direct Use for FFP

The documentations requirement for LMO-FFP have been extremely controversial as countries had different views regarding specific identification requirements to be included in the documentation. It was agreed that the documentation can mention “may contain” LMOs where identity of the LMO is not known. Further details have been elaborated in “Curitiba Rules” agreed in COP-MOP3 after intense negotiation in Curitiba, Brazil in 2006. Documentation accompanying LMOs-FFP, in commercial production and authorised in accordance with domestic regulatory frameworks, is to be in compliance with the requirements of the import and clearly state:

- When the identity of the LMO is known through means such as identity preservation systems, document should clearly mention "Contains LMOs-FFP".
- When the identity of the LMO is not known through means such as identity preservation systems; the shipment "may contain one or more LMO - FFP".
- The LMOs are not intended for intentional introduction into the environment.
- Specification of the identity of LMOs viz., its common, scientific and where available, commercial names of LMOs.
- Transformation event, code of LMOs.
- Any unique identification, if available for accessing information in BCH.
- A contact point for further information.
- The internet address (website) of the Biosafety Clearing-House (BCH) for further information.

It was agreed that the expression "may contain" does not require a listing of LMOs of species other than those that constitute the shipment.

4.2. Article 18 (2b): Documentations Requirement for LMOs Destined for Contained Use

- Clearly identifies content as LMOs including common and scientific names of organisms and as "destined for contained use"
- Specifies any requirements for the safe contained use, handling, storage, transport and use of LMOs. In case there is no such requirement, indicate that there is no specific requirement.
- Name and address of the consignee, and exporter or importer (contact point for further information, including name and address of individual/ institution to whom the LMOs are consigned) and the contact details necessary to reach them as far as possible in case of emergency.
- Provides further information, where appropriate such as the commercial name of the LMOs, new or modified traits, transformation events, risk class, specification of use.
- Any unique identification as a key for accessing information in the BCH.

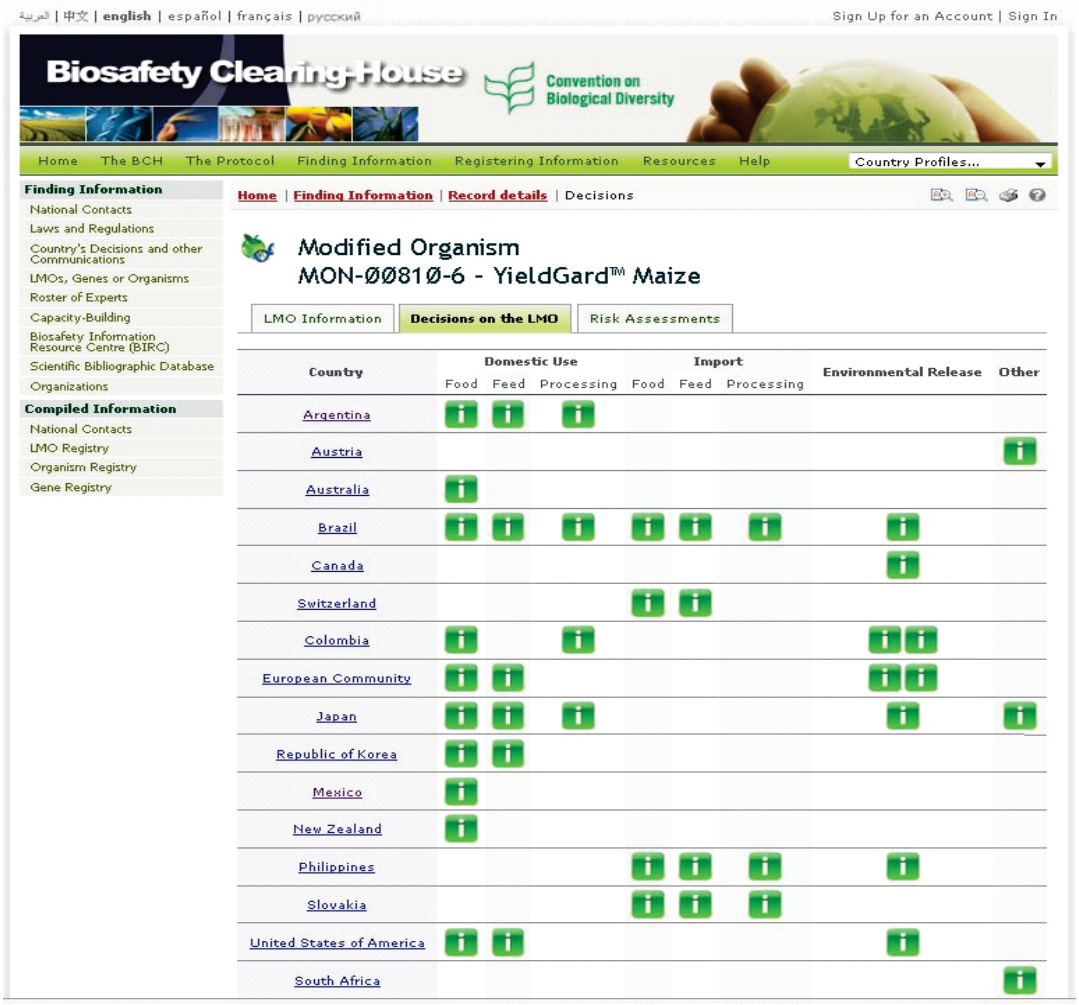
4.3. Article 18 (2c): Documentations Requirement for LMOs Intended for Intentional Introduction into the Environment of the Party of Import

- Clearly identifies content as LMOs.
- Specific identity of LMOs including common and scientific names, relevant traits and/or genetic modification including transgenic traits, characteristics such as transformation event (s) or reference to a system of unique identification.
- Gives any requirement for the safe handling, storage, transport and use. In case, there is no specific requirement, it is also to be indicated.
- Contains the name and address of the importer and exporter.
- Provides a contact point for further information including an individual or organisation in possession of relevant information in case of emergency.
- Includes a Declaration that movement of the LMOs is in conformity with the requirements of this Protocol applicable to the exporter.
- Provides further information, where appropriate e.g. commercial name, risk class and import approval for first transboundary movement of the LMO.

4.4. Unique Identifiers

Documentation requirements for all three categories of LMOs require reference to a unique identifier code. Till date, only one unique identification system exists i.e. *OECD Unique Identifiers for Transgenic Plants*. Developers of transgenic plants assign the unique identifier to each living modified plant that is approved for commercial use. It is a 9-digit code composed of 3 elements separated by dashes; 2 or 3 alphanumeric digits designate the applicant; 5 or 6 alphanumeric digits to designate the transformation event; and 1 numerical digit for verification. (Example: MON-00810-6 is the unique identifier for Monsanto's YieldGard maize, a type of maize that has been modified to be resistant to a certain insect, the European corn borer). Unique identifier can be used to search BCH for information about specific LMOs.

There may not be a stand-alone document to accompany shipments of LMOs. Instead, the information that is to accompany shipments of LMOs is included in existing types of shipping documentation, e.g. invoices, bills of lading, way bills, etc.



5. Unintentional and Illegal Transboundary Movement

In addition to transboundary movement of LMOs, the CPB also contains provisions on Unintentional transboundary movements and emergency measures (Article 17) and Illegal transboundary movements (Article 25).

5.1. Unintentional Transboundary Movement

Unintentional transboundary movement is a transboundary movement of a LMO that has inadvertently crossed the national borders of a party where LMO was released and the requirements of Article 17 of the Protocol apply to such transboundary movements only if the LMO involved is likely to have significant adverse effects on the conservation and sustainable use of biological diversity also taking into account the risks to human health, in

the affected or potentially affected states. Parties are required to notify the BCH and the potentially affected States. Each party is required to specify a contact point for the purpose of receiving such notifications. Parties under whose jurisdiction such releases occur are also required to consult potentially affected States to determine appropriate responses, including emergency measures.

5.2. Illegal Transboundary Movement

Illegal Transboundary Movement (Article 25) is a transboundary movement of LMOs carried out in contravention of the domestic measures to implement the Protocol that have been adopted by the party concerned.

Parties are required to adopt domestic measures to prevent and if appropriate, penalize transboundary movement of LMOs that occur in contravention of its domestic measures to implement the protocol. Such movements are deemed as illegal transboundary movements. In the case of such illegal transboundary movement, the affected party may request the Party of origin to dispose, at its own expense, of the LMO in question by repatriation or destruction, as appropriate.

Each Party is required to make available information concerning cases of illegal transboundary movement to BCH.

6. Documentation Accompanying Shipments of LMOs: A Case-study on Existing Documentation Systems for LMOs Destined for Contained Use in India

- ICAR- National Bureau of Plant Genetic Resources (NBPGR) is the nodal organisation for management of plant genetic resources (PGR) including transgenics. It facilitates exchange of germplasm through proper implementation of quarantine measures. It is vested with the authority to issue Import Permit (for import) and Phytosanitary Certificate (for material meant for export) and undertake quarantine processing of all seed material and plant propagules of germplasm including transgenic planting material exchanged for research purposes.

- In India, the import of agricultural commodities including germplasm is made as per the provisions of Plant Quarantine (Regulation of Import into India) Order 2003 and its Amendments ((herein after referred to as the PQ Order 2003) promulgated by Ministry of Agriculture and Farmers' Welfare, Government of India. This Order ensures the incorporation of "Additional/ Special Declarations" for import of commodities free from quarantine pests, on the basis of pest risk analysis (PRA), particularly for seed/ planting material (http://www.plantquarantineindia.org).

For import of transgenic plant material, for research purpose, ICAR-NBPGR is the nodal institute. The Recombinant Biosafety Guidelines, 1990 stipulate detailed procedure for import including the type of containment, packaging, labelling, contact point and documents to accompany shipment. For importing transgenic planting material both by public and private sector for R&D purposes, technical clearance for import is sought by the indenter through Institutional Biosafety Committee (IBSC) from Review Committee on Genetic Manipulation (RCGM), Department of Biotechnology (DBT), Ministry of Science and Technology. After getting the technical clearance from RCGM, request is made to the Director, ICAR-NBPGR for issuance of Import Permit as per the regular procedure. All transgenic plant material received for research purposes are tested by ICAR-NBPGR for the pests of quarantine significance and absence of GURT technology which is banned in India. The import of a transgenic plant material is required to be accompanied by an appropriate Phytosanitary Certificate issued by the Competent Authority of the country of export.

For import of plant germplasm/ transgenics/GMOs for research/experimental purpose by the public/private organisations, the importer makes an application for import to the Director, ICAR-NBPGR, New Delhi in the prescribed proforma PQ Form 08 and the Import Permit is issued by the Director, ICAR-NBPGR, New Delhi in the PQ Form 09.

The documents which are essentially required for the consignment of LMOs for contained use (Anonymous, 2017; Bhalla *et al*., 2014; 2009) are given below:

- Information to IBSC/ RCGM for import/ exchange of GMOs and products thereof for research purpose (Annexure-I)
- **Import Clearance:** Permit letter for authorization to import genetically modified organisms (LMOs) and products thereof for research and development purposes issued by RCGM of DBT, Ministry of Science and Technology (Annexure II/sample)
- Application for Permit to import germplasm/ transgenics/ genetically modified organisms for research purpose – **PQ Form 08** (Annexure III)
- Permit for Import of germplasm/ transgenics/ genetically modified organisms for research purpose – **PQ Form 09** (Annexure IV)
- **Import Permit** (IP) is issued by the Director, ICAR-NBPGR, New Delhi as per the PQ Form 09 of the PQ Order 2003 only after RCGM has accorded the import clearance and the importer has submitted the required undertakings/ certificates. It gives the details of the material and additional declaration that the material should be free from the specific pests as specified in the Schedules V and VI of the PQ Order 2003.

- IP gives the details of material i.e. common and scientific name; Country of origin; Name and address of the consignee etc.; Number of samples; Type of material-trial/ germplasm etc.; Transgene (s)/ trait

- **Red/White colour tag** for Transgenic import in PQ form 11 (Annexure-V).

- **Phytosanitary Certificate** issued by the Govt. official of country of origin giving the details of material and treatment in the model format prescribed under the International Plant Protection Convention (IPPC) of FAO. Issue of Phytosanitary Certificate for material under export in the PQ Form 21, which is as per IPPC format (Annexure-VI).

- **Commercial invoice** giving details of the material from the country of export (Annexure-VII/sample).

- Declaration for the absence of embryogenesis deactivator gene.

Presently India has experience in importing LMOs for contained use only. To this extent adequate legal and administrative measures are in place. While there is a legal provision for documentation requirement for LMOs- FFP and intentional release, the required administrative coherence and capacity for detection among the various State actors is lacking mainly due to absence of any experience or need in importing such products. The bulk shipment(s) of transgenic plants or plant products or GMOs to be dealt as per the provisions of the Rules for manufacture, use, import, export and storage of hazardous micro-organisms, genetically engineered organisms or cells made under Sections 6, 8 and 25 of the Environment (Protection) Act, 1986 (29 of 1986) or under the mechanism established as per the provisions of CPB by the MoEF&CC.

However, in view of the enhanced global trade in GMOs, there is an urgent need to address this issue as the Protocol also requires Parties to take sound measures in their domestic regulations to address the issue of unintentional transboundary movement (Article 17) and illegal transboundary movements (Article 25) of LMOs. The documentation requirement therefore, needs to be supported with adequate legal backing, guidelines on the roles and responsibility of various agencies, infrastructure and trained personnel for identification of LMOs.

7. References

- Anonymous (2017) *Guidelines for Import and Quarantine of Transgenic Planting Material*. ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. 40+iv p.

- Bhalla Shashi, Chalam VC, Tyagi V, Lal A, Agarwal PC and Bisht IS (2014) *Teaching Manual on Germplasm Exchange and Plant Quarantine.* National Bureau of Plant Genetic Resources, New Delhi, India. 340+viii p.
- Bhalla Shashi, Chalam VC, Lal A and Khetarpal RK (2009) *Practical Manual on Plant Quarantine*. National Bureau of Plant Genetic Resources, New Delhi, India. 180 p.
- The Cartagena Protocol on Biosafety. http://www.biodiv.org/biosafety Retrieved October 18, 2017.
- Plant Quarantine (Regulation of Import into India) Order 2003,Union Ministry of Agriculture and Farmers' Welfare, Govt. of India, vide Notification No. S.O.No.1322 (E), dated 18th November, 2003. http://www.plantquarantineindia.org Retrieved November 8, 2017.

Annexure-I

Information to IBSC/ RCGM for Import/ Exchange of GMOs and Products thereof for Research Purpose

1. Name of the Applicant

   Designation

   (a) Address (Registered Office)

   Telephone No.

   Telex No.

   Fax No.

   e-mail

   (b) Address (Research Station)

   Telephone No.

   Telex No.

   Fax No.

   e-mail

2. Application for (to indicate the purpose):

3. Objectives of the proposal:
   (Applicant should also indicate the relationships of the work plan with environmental safety issues, taking al'1o into consideration the safety to human and animal health when open field experiments are parts of objectives).

4. Description of the GMOs/ product there of (in scientific terms):
   (a) Morphology
   (b) Physiology
   (c) Number of copies of the genes incorporated
   (d) Status of approval is country of origin.

5. Quantity of GMOs/ products there of to be imported/exchanged:

6. Summary of the proposed work plan utilizing GMOs/ products there of:
   (This should indicate schematic lab work, green house studies whenever applicable and details of open field experiments including the map of the experimental plot(s) & the planting pattern of trans gene plants! seeds)

7. Details on:
   (a) Source of nucleic acid(s):

(b) Nucleic acid sequence (Please enclose the nucleic acid sequence map of the target gene):
(c) Vector(s) (Please enclose the map of the vector gene):
(d) Sequence of the genes incorporated/ to be incorporated into the host organism.
(e) Host(s) that carrying the vector(s)/ target gene(s):
(f) Manipulative procedures in outline:

8. Source of GMOs/products there of:

   Name of the Agency

   Contact person's name

   Address

   Telephone No.

   Telex No.

   Fax No.

   e-mail

9. Mode of shipment:

10. Decontamination, disposal mechanisms & risk management:

11. Any other relevant points(s)

12. Declaration:

    I declare that the information provided in the above format is correct and accurate to the best of my knowledge. The "Safety Guidelines" brought out by the Department of Biotechnology, Ministry of Science & Technology, Govt. of India will be and is being strictly followed. The imported/ exchanged material will be and is being utilized for the said purpose only. In case any untoward incident occurs, the Chairman of the IBSC and the Member-Secretary of the RCGM will be informed immediately.

Date: Signature of the Applicant

Forwarded The proposal set out above has been considered by the "Institutional Biosafety Committee" on _____ and is forwarded to RCGM for further necessary action.

Date : Signature of the Chairman, IBSC

(Note : Please submit 20 copies of the application to the Department of Biotechnology for placing the same in the meeting of RCGM)

Annexure II
(Sample)

Permit Letter for Authorization to Import Genetically Modified Organisms (LMOs) and Products thereof for Research and Development Purposes - Import Clearance issued by RCGM of DBT, Ministry of

भारत सरकार
विज्ञान और प्रौद्योगिकी मंत्रालय
बायोटेक्नोलॉजी विभाग
GOVERNMENT OF INDIA
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

सत्यमेव जयते

स्वच्छ भारत
एक कदम स्वच्छता की ओर

ब्लाक-2, 7वां तल, सी0 जी0 ओ0 कम्पलेक्स
लोधी रोड, नई दिल्ली-110003
Block-2, 7th Floor, C.G.O. Complex
Lodhi Road, New Delhi-110003

PERMIT LETTER FOR AUTHORIZATION TO IMPORT GENETICALLY MODIFIED ORGANISMS (GMOs)/LIVING MODIFIED ORGANISMS (LMOs) AND PRODUCTS THEREOF FOR RESEARCH AND DEVELOPMENT PURPOSE

PERMIT NUMBER: BT/BS/17/219/2006-PID **DATE OF ISSUE: 09.03.2017**
DATE OF EXPIRY: 08.03.2019

Permittee

Name:	Bindu Nair,
	Lead-Molecular Breeding Technology,
Organization:	Monsanto Research Centre
Address:	(a unit of Monsanto Holdings Pvt. Ltd.)
	Ground Floor, Green heart Phase IV,
	MFAR, Manyata Tech Park, Rachenahalli Village,
	Nagawara, Bangalore-560045,
Tel:	+91 80 30011680
E-mail:	mhpl.regulatory@monsanto.com
IBSC Code:	MHPL

Subject: Application submitted by Monsanto Research Centre, M/s. Monsanto Holdings Pvt. Ltd., Bangalore for permission to import 50 gm each of transgenic Corn and Cotton lyophilized leaf tissues from M/s. Monsanto Company, USA for R&D purpose.

AUTHORISATION: In accordance with the Allocation of Business Rules 1961 of Government of India, as notified vide Notification No. CD-172/86 dated 27.02.1986 and Notification No. CD-87-87 dated 31.01.1987 and the powers conferred through the Sections 6,8 and 25 of the Environment (Protection) Act, 1986 read along with the Central Government Gazette Notification No. GSR 1037(E), dated 5.12.1989 issued by the Ministry of Environment and Forests, New Delhi and based on the Simplified Procedures/ Guidelines for Import/Export/ Exchange of Genetically Engineered Organisms and Product(s) thereof for Research Purpose issued by the Department of Biotechnology vide on No. BT/BS/17/635/2015-PID dated 22.09.2015, authorization is accorded to **Monsanto Research Centre, M/s. Monsanto Holdings Pvt. Ltd., Bangalore** to import **50 gm each of transgenic Corn and Cotton lyophilized leaf tissues** from **M/s. Monsanto Company, USA** for research and development purposes with properties indicated below, subject to the conditions mentioned in this letter.

PERIOD: The permit letter shall be in force from **09.03.2017** to **08.03.2019** unless it is sooner suspended or cancelled under the said Rules.

DESCRIPTION OF GMOs/LMOs/MATERIALS:

1. **GMOs/LMOs/material(s) to be imported:** Transgenic Corn and Cotton lyophilized leaf tissues as per details given at S. No. 2

2. **Quantities of the material(s) to be imported:**

Crop	Breeding stacks	Quantity
Cotton	MON 531 x MON 15985 x MON 88913	50 grams
	MON 531 x MON 88913	50 grams
	MON 531 x MON 15985 x MON 88913 x COT 102	50 grams
Corn	MON 89034 x NK 603 x MON 810	50 grams
	MON 89034 x NK 603	50 grams
	MON 810 x NK 603	50 grams

Website : http://www.dbtindia.nic.in http://www.btisnet.gov.in
दूरभाष/Telephone : 24363012, 24362329 फैक्स/Fax : 011-24362884

3. Purpose: For Research and Development

4. Source of material(s) (Name, Organization, address):

Dr. Jennifer Depp,
M/s. Monsanto Company,
800 North Lindbergh Blvd. Mail Stop
BB4926-B, St. Louis, MO 63141, USA
Email: jennifer.l.depp@monsanto.com

5. Type of permit:

The permission is granted for import of **50 gm each of transgenic Corn and Cotton lyophilized leaf tissues** by **Monsanto Research Centre, M/s. Monsanto Holdings Pvt. Ltd., Bangalore** within a period of **two years.**

6. Instructions for use:

i. No GMOs/LMOs are allowed for experimentation for commercial production/manufacturing of the product without prior authorization from the Competent Authority.
ii. All rDNA materials are to be destroyed and disposed of in accordance with the Recombinant DNA Safety Guidelines, 1990 of the Government of India after conclusion of the experiments.
iii. All experiments to be carried out are to be documented.
iv. The applicant is directed to submit fresh application to RCGM with the approval of IBSC for information to carry out R & D work on **transgenic Corn and Cotton lyophilized leaf tissues** and would submit the 'statement of utilization of the imported **materials** to this Department.

7. Condition(s) of issuance: **As above**

8. Mode of Transport:

□ Rail □ Road √ Air □ Ship

9. Handling and packing instructions:

The GMOs/LMOs/Material(s) mentioned herein shall be handled, packaged and transported as specified in "r-DNA Safety Guidelines-1990" of the Department of Biotechnology, Government of India.

Kindly acknowledge the receipt of the letter.

(S. R. Rao)
Member Secretary, RCGM &
Scientist-G, DBT

Copy for information to:

1. The Chairman, GEAC, Ministry of Environment and Forests, Indira Paryavaran Bhawan, Ali Ganj, Jor Bagh Road, New Delhi-110003.
2. Dr. Jennifer Depp, M/s. Monsanto Company, 800 North Lindbergh Blvd. Mail Stop BB4926-B, St. Louis, MO 63141, USA
3. Office copy for file.
4. Guard file.

Signature & title of issuing officer:

(S. R. Rao)
Member Secretary, RCGM &
Scientist-G, DBT

Annexure III

PO Form 08

Application for Permit to Import Germplasm/ Transgenics/ Genetically Modified Organisms (GMO's)
NATIONAL BUREAU OF PLANT GENETIC RESOURCES
INDIAN COUNCIL OF AGRICULTURAL RESEARCH NEW DELHI, INDIA

(For Research Purpose)

To
The Director,
National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi-110012

I hereby apply for a permit in accordance with provisions of clause 6 (2) of the Plant Quarantine (Regulation of Import into India) Order, 2003 issued under the Sub-section (1) of Section (3) of the Destructive Insects & Pests Act, 1914 (2 of 1914), authorizing the import of plants/planting materials for research purposes as per details given below:

1. Name and address of the applicant :
2. Research and Development (R&D) status /affiliations of the organisation [Please attach relevant documents] :
3. Exact description of Seeds/Planting Materials to be imported :
   (a) Common and botanical name
   (b) Germplasm/ variety/ hybrid/ composite/ synthetic/ clone/ provenance/ others :
   (c) Form of material required (seed /rooted plants/ scions/ tubers/ cuttings/ bulbs *in vitro* cultures :
   (d) Parentage, if known :
4. Place of collection/origin of material to be imported (country/state) :
5. Whether transgenic/GMO or not? [If yes, attach the approval letter issued by RCGM (DBT) in original] :

6. Name and address of the organization / institution producing the material :

7. Number of samples to be imported :

8. Quantity to be imported (separately for each accession/ variety/ hybrid/ transgenic/ GMO) :

9. Suggested source of availability of material including published reference, if known :

10. (a) Whether the aforesaid germplasm/ variety/ hybrid was imported by you earlier? If so, details thereof (year, quantity, source, etc.) :

    (b) Was the material shared with other scientists/ National Gene Bank at NBPGR? :

11. Expected date and arrival in India :

12.. Mode of shipment (Airmail/ Air freight! accompanied baggage) :

13. Place where imported seeds/ planting material will be grown and scientists under whose supervision the seeds/ planting material will be grown :

Declaration

1. I hereby declare that the germplasm under import has no commercial value/ exclusive ownership and may be shared freely for research purposes.
2. The germplasm does not contain any terminator genes or terminator technology (TT) or genetic usage restrictive technologies (GURTs).
3. I undertake that the material is exclusively for research purposes.

Place:
Date: Signature of the Applicant & Address

Please Note: For further information on import permit issuance fee, consignment handling charges and quarantine fee, please see our website http:// www.nbpgr.emet.in

Contact: Tel. No. 91-11-25843697 or Fax. 91-11- 25842495 or E-Mail: director.nbpgr@icar.gov.in

Annexure IV

PO Form 09

ICAR-National Bureau of Plant Genetic Resources
New Delhi 110012

Permit for Import of Germplasm/ Transgenic/ Genetically Modified Organisms for Research Purpose

Permit No. _____ Date of issue _____

Valid up to _____

In accordance with the provisions of clause 6 (2) of the Plant Quarantine (Regulation of Import into India) Order 2003 issued under Sub-section (1) of Section 3 of the Destructive Insects & Pests Act,1914, I hereby grant permission to import of germplasm/transgenic/genetically modified .organisms herein specified

1. Name and address of importer

2. Name and address of exporter

3. Country of origin

Point of Entry

4. Description of germplasm/ transgenic/Genetically modified organism (Botanical name)	5. Variety to be imported	6. Quantity (Weight/Nos.)	7. No of Pakages	8. Mode of Packing

9. The above permission is granted subject to following conditions:-

(1) The consignment of germplasm/transgenic shall be free from soil, weed species and plant debris.

(2) (i) The consignment shall be accompanied by a Phytosanitary Certificate/Phytosanitary Certificate (re-export issued by an authorized officer in the country of origin /country of re-export) as the case may be with additional declaration for the freedom from:

a) _____

b) _____ or that the above specified pests do not occur in the country or state of origin.

(ii) Certified that the germplasm/transgenic as described above obtained from mother crop/ stock which were inspected on regular intervals by an appropriate authority in the country of origin and found free from:

(3) The consignment shall be grown in an approved post-entry quarantine facility established by the importer at_____ (name of location of PEQ facility) under the supervision of _____ for a period of (days/months)_____ (Name & Address of Inspection Authority)

(4) The permit is not transferable and valid for one-time import. The permit number shall be quoted on the Phytosanitary Certificate issued at the country of origin or re-export as the case may be.

Place: New Delhi Seal Name

Date: Signature
Director
ICAR-National Bureau of Plant Genetics Resources

Annexure V
PQ Form 11

RED / WHITE COLOUR TAG

Permit Number _____

Valid up to _____

This package contains: Transgenic lines of plants/ genetically engineered microorganisms.

Do not open except at the bio-safety laboratory in the presence of Research Scientist and Plant Quarantine Authority.

RUSH AND DELIVER TO

Officer-in-charge, Plant Quarantine Station,_____

For onward transmission to Bio-safety lab.

REVERSE OF TAG

Directions for Mailing transgenic lines of plants/ genetically engineered or modified microorganisms:

Under this tag or label only materials covered under the Permit should only be shipped and any other material shall be confiscated and destroyed. The packaging should be confirmed with bio-safety regulations. The inner container should carry name and description of the transgenic line or microorganism and should be hermetically sealed. The outer container shall carry the Consignee's name and address and the Invoice and placed inside secured package. Paste Red/White label on the face of each package.

Do not write anything on the label. Do not place any delivery address outside package. Write the foreign shipper's name on outside of package and full postage.

Annexure-VI

Phytosanitary Certificate (Exporting Country)

No phytosanitary certificate can be issued until an application is completed (7 CFR 353)

FORM APPROVED
OMB NO. 0579-0052

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

PHYTOSANITARY CERTIFICATE

FOR OFFICIAL USE ONLY

PLACE OF ISSUE
Cedar Rapids, Iowa

NO.
F-S-19113-03175398-7-N

DATE INSPECTED
May 21, 2013

TO: THE PLANT PROTECTION ORGANIZATION(S) OF
India

CERTIFICATION

This is to certify that the plants, plant product or other regulated articles described herein have been inspected and/or tested according to appropriate official procedures and are considered to be free from the quarantine pests, specified by the importing contracting party and to conform with the current phytosanitary requirements of the importing contracting party including those for regulated non-quarantine pests.

DISINFESTATION AND/OR DISINFECTION TREATMENT

1. DATE	2. TREATMENT
**********	**********
3. CHEMICAL (active ingredient)	4. DURATION AND TEMPERATURE
**********	**********
5. CONCENTRATION	6. ADDITIONAL INFORMATION
**********	**********

DESCRIPTION OF THE CONSIGNMENT

7. NAME AND ADDRESS OF THE EXPORTER	8. DECLARED NAME AND ADDRESS OF THE CONSIGNEE
Monsanto Company 503 South Maplewood Avenue Williamsburg, Iowa 52361	MONSANTO INDIA LTD., 2nd Floor, F block, International Trade Tower ,Nehru Place, New Delhi 110019 India
9. NAME OF PRODUCE AND QUANTITY DECLARED	10. BOTANICAL NAME OF PLANTS
(1) .7 Kilograms Corn (Seeds) **********	(1) Zea mays **********
11. NUMBER AND DESCRIPTION OF PACKAGES	12. DISTINGUISHING MARKS
(1) 2 Boxes **********	(1) None **********
13. PLACE OF ORIGIN	14. DECLARED MEANS OF CONVEYANCE
(1) Hawaii, USA **********	Air Freight
	15. DECLARED POINT OF ENTRY
	New Dehli

WARNING: Any alteration, forgery, or unauthorized use of this phytosanitary certificate is subject to civil penalties of up to $250,000 (7 U.S.C. Section 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. Section 1001).

ADDITIONAL DECLARATION

Import Permit Number 247/2013 was presented. The seed in this shipment is free from Maize chlorotic mottle virus and wheat streak mosaic virus.

NAPPO

Page 1 of 1

16. DATE ISSUED	17. NAME OF AUTHORIZED OFFICER *(Type or Print)*	18. SIGNATURE OF AUTHORIZED OFFICER
May 21, 2013	Nicolas A. Groves	

No liability shall attach to the United States Department of Agriculture or to any officer or representative of the Department with respect to this certificate.

PPQ Form 577 FEB 2001 Previous editions are obsolete after 6/30/01

SHIPPER'S ORIGINAL

Annexure-VII
(Sample)

Commercial Invoice (Exporting Country)

MONSANTO

Commercial Invoice

Monsanto Company 800 N Lindbergh Blvd St. Louis, MO 63167, US SHIP FROM: 503 S MAPLEWOOD AVE WILLIAMSBURG,IA,52361,US	**Invoice Number** 809404159	**Page Number** 1/2
	Date 01 APR 2013	**Order Number** 803797085
	Customer Purchase Order IA_IN_CORN_2013	**Customer Number** 3114566
Ship-to or Consignee: 1777689 NATIONAL BUREAU OF PLANT GENETIC RESOURCES INDIAN AGRICULTURAL RESEARCH INSTITUTE CAMPUS, PUSA 110012 NEW DELHI INDIA	**Bill-to 3114566** MONSANTO INDIA LTD 2 ND FLOOR , F BLOCK INTERNATIONAL TRADE TOWER NEHRU PLACE 110019 NEW DELHI INDIA	
Forwarding Agent or Notify 270028 FEDEX PO Box 1140 MEMPHIS TN 38101-1140	**Customs Doc**	**Insured Value**
	Country Shipped From US	**Country of Destination** India
Shipment Date 01 APR 2013 / **Shipped From** WILLIAMSBURG IA	**Terms of Delivery** CPT New Delhi ZNC- No charge	
Carrier FEDEX		
Vessel\ Aircraft / **Port of Loading**		
Port of Discharge / **Place of Delivery** NEW DELHI, India		

Item	Goods Description	Quantity	Unit Price	Amount
1	11297979 **MON-89Ø34-3 x MON-ØØ6Ø3-6 (NK603)** **LEPIDOPTERAN RESISTANT, GLYPHOSATE HERBICIDE TOLERANT CORN SEED FOR RESEARCH PURPOSES ONLY** **LIVING MODIFIED ORGANISM (LMO)** **PFPGJ-1-66-1 to 5** **PFPGJ-2-66-1 to 5** **PFPGJ-1-149-1 to 5** **PFPGJ-1-203-1 to 5** **PFPGJ-2-203-1 to 5** Customs Value Country of Origin : Hawaii, USA Commodity Code: 1005.10.0010 Net Wt. = 0.50 KG	0.50 KG	3.15 USD/ per KG	1.58
2	11297973 **MON-ØØ6Ø3-6 GLYPHOSATE HERBICIDE TOLERANT CORN SEED FOR RESEARCH PURPOSES ONLY** **LIVING MODIFIED ORGANISM (LMO)** **PFHTT-1-94-1 to 5** **PFHTT-1-107-1 to 5** Customs Value Country of Origin : Hawaii, USA Commodity Code: 1005.10.0010 Net Wt. = 0.20 KG	0.20 KG	3.15 USD/ per KG	0.63

These commodities, technology and/or software, were exported from the United States in accordance with Export Administration Regulations. Diversion contrary to U.S. law prohibited.

MONSANTO

Commercial Invoice

Monsanto Company 800 N Lindbergh Blvd St. Louis, MO 63167, US SHIP FROM: 503 S MAPLEWOOD AVE WILLIAMSBURG,IA,52361,US	**Invoice Number** 809404159	**Page Number** 2/2
	Date 01 APR 2013	**Order Number** 803797085
	Customer Purchase Order IA_IN_CORN_2013	**Customer Number** 3114566
Ship-to or Consignee: 1777689 NATIONAL BUREAU OF PLANT GENETIC RESOURCES INDIAN AGRICULTURAL RESEARCH 110012 NEW DELHI INDIA	**Bill-to 3114566** MONSANTO INDIA LTD 2 ND FLOOR , F BLOCK NEHRU PLACE 110019 NEW DELHI INDIA	

Item	Goods Description	Quantity	Unit Price	Amount
	NOTIFY PARTY: DLC Forwarders Pvt Ltd N-10, 1st Floor, Kalka Ji New Delhi-110019 Tel# 41605234, Fax# 41605235 WE CERTIFY THIS INVOICE TO BE TRUE AND CORRECT, MONSANTO COMPANY AUTHORIZED SIGNATURE, INTERNATIONAL DISTRIBUTION PHYTO #: F-S-19113-03175398-7-N IMPORT PERMIT #: 247/2013 AWB NO.: 7998 1219 0262 Total Gross Weight = 1.9 KG Total Net Weight = 0.70 KG		Total USD	2.21 For customs valuation only. No commercial value.

Use of Biosafety Clearing-House: An Information Portal on Living Modified Organisms

8

Vibha Ahuja
Biotech Consortium India Limited, New Delhi 110002, India
Email: *vibhaahuja.bcil@nic.in*

1. Introduction

The Biosafety Clearing-House (BCH) is an information exchange mechanism set up under the Cartagena Protocol on Biosafety (CPB) with two main objectives: it provides a platform to exchange information and experience with living modified organisms (LMOs); and it also assists governments that have ratified to implement the Protocol (referred to as Parties) to comply with their obligations. Article 20 of the CPB (Box 1) provides for establishment of BCH as part of the clearing-house mechanism of the CBD.The BCH provides open and easy access to a variety of scientific, technical, environmental, legal and capacity building information. The central portal of the BCH is available on web at https://bch.cbd.int/. The information is available in all six languages of the UN viz., Arabic, Chinese, English, French, Russian and Spanish.

Box 1: Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity (CBD) is an international agreement governing the movement of living modified organisms (LMOs) resulting from modern biotechnology from one country to other. The term LMO is defined in the CPB as a living organism that possess a novel combination of genetic material obtained through the use of modern biotechnology. The CPB was adopted in 2000 and entered into force in 2003. As on date, 171 countries are Party to the CPB. India is also a Party to the CPB. Article 20 of the CPB broadly lists the specific requirements that each Party must provide to BCH; requirement to post information on BCH is referred to in other articles of the CPB also.

The BCH is essential for the successful implementation of the CPB. It assists Parties and other stakeholders in different ways in the implementation of the CPB. For example, it provides a "one-stop shop" where users can readily access or contribute relevant biosafety-

related information. This would assist Governments to make informed decisions regarding the importation or release of LMOs.

The BCH consists of a central portal with linkages to distributed network of national, regional and international nodes/databases. The central portal is a gateway to all sections of the BCH including the search pages. Parties have been provided with national nodes to provide country specific information on their respective portals, which are inter operable with central portal.

2. Categories of Information

Each Party to the CPB is required to designate one National Focal Point (NFP), who is responsible for registering national information in prescribed formats. As the focus of CPB is on transboundary movement of LMOs, BCH enables governments to inform others about their final decisions regarding the import of LMOs. In addition, the BCH contains information on national laws, regulations and guidelines for implementing the Protocol, summaries of risk assessments and environmental reviews, database, reports on relevant issues etc. Governments that are not Parties to the Protocol are also encouraged to contribute information to the BCH, and in fact a large number of the decisions regarding LMOs have

been registered in the BCH by non-Party governments. Various categories of information available on BCH and steps to access the same are explained in following sections.

Information currently available on BCH is organized into National Records and Reference Records:

- **National Records** are submitted by Governments as mandated by Article 20 of the Protocol.
- **Reference Records** are submitted by general BCH users. These also include three registries regarding LMO information appearing in other BCH records *viz.*, LMO registry, Gene registry and Organisms registry.

There are common formats prescribed by the CBD Secretariat for submission of the information to ensure uniformity. The use of standardized terminology to categorize the information within the databases, allows many users of BCH to use the same terms whether they are registering information or searching for it. Records submitted by the BCH NFP or National Authorized Users (NAU) and validated by BCH-NFPs are treated as National Records while records submitted by other users and validated by the Secretariat are treated as Reference Records. Table 1 provides the list of type of records under each category.

Table 1. Information available on BCH

National records	Reference records
National Contacts i. National Focal Points ii. Competent National Authorities iii. National Biosafety Websites and Databases	**LMOs, Genetic Elements or Organisms** i. The LMO-UID Registry ii. Gene Registry iii. Organism Registry
Laws and Regulations i. National Laws, Regulations ii. Guidelines and Bilateral, Regional and Multilateral Agreements	**Capacity-Building** i. Biosafety Capacity Building Projects ii. Capacity-Building Opportunities iii. Compendium of Academically-Accredited Biosafety Courses iv. Capacity-Building Needs and Priorities
National Reports National reports and analysis	**Directory of International Organisations involved in Biosafety Activities** International organisations involved in

	activities relevant to implementation of the Biosafety Protocol with summaries of their activities and contact information
Country's Decisions and other Communications i. Decisions on LMOs under Advance Informed Agreement ii. Decisions on LMOs for food, for feed or for processing under Article 11 iii. Other decisions & declaration iv. Risk Assessment Reports	**The BCH Virtual Library** i. Biosafety Information Resource Centre (BCH-BIRC) ii. Scientific Bibliographic Database on Biosafety (Bibliosafety). iii. Access to Research4Life, a collective name for four public-private partnerships to provide the developing world with free or low cost online access to academic and professional peer-reviewed content.
Roster of Experts Access a database of experts in biosafety, searchable using various fields.	

All national records are submitted/validated by NFPs to BCH, designated by Parties to the CPB. Reference Records are submitted by any users and validated by the Secretariat. Information that a Party needs to make available to the BCH is an ongoing process. The main categories of information that Parties must register in the BCH are described below:

2.1. National Contacts

The CPB requires each Party to designate National Focal Points (NFPs) and Competent National Authorities (CNAs) to fulfil its obligations under the CPB.

NFP to CPB is the primary point of contact for all information about the CPB in a country and responsible for liaising with the CBD Secretariat. NFPs to BCH are responsible for liaison with the Secretariat on technical issues related to the BCH as per Article 20 of the CPB and subsequent decisions.

CNAs are responsible for performing the administrative functions required by the Protocol, including handling of notifications/applications and communicating to the notifier/ applicant and to the BCH decisions regarding importation or release of LMOs. Parties can designate one or more CNAs for various administrative functions. In such cases, the

information provided through the BCH should, at a minimum, specify which competent national authority is responsible for which type of LMO.

The section on national contacts provides names and addresses of CNAs designated by each Party as required by the Article 19 of the CPB. Compiled list of all national contacts and competent national authorities is also available at https://bch.cbd.int/database/compiled-national-contacts.

Parties are required to designate National point of contact for receiving notifications regarding unintentional transboundary movements of LMOs and emergency measures as required by the Article 17 of the CPB. This section provides links to national websites and/or databases, maintained by Parties to CPB that are relevant to the implementation of the CPB.

2.2. National Laws and Regulations

Parties to CPB register all existing laws, regulations and guidelines for implementation of the CPB. Information requirements by Parties for seeking various types of LMOs such as for direct use as food or feed, or for processing (LMOs-FFP) are also included (Box 2).

Box 2: Procedures for Transboundary Movement of LMOs in CPB

Practical rules and procedures have been established under the CPB for the safe transfer, handling and use of LMOs with specific focus on regulating transboundary movement of LMOs. Categories of LMOs covered under the Protocol are broadly divided into three categories.

- LMOs for intentional introduction into the environment (e.g. seeds, live fish)
- LMOs intended for direct use as food or feed, or for processing, LMPOs-FFP (e.g. agricultural commodities – corn, canola, cotton)
- LMOs for contained use (e.g. bacteria for laboratory scientific experiment)

Specific procedures have been defined for LMOs for intentional release and LMOs-FFP. The Advance Informed Agreement (AIA) procedures applies to the first intentional transboundary movement of LMOs for intentional introduction into the environment of the Party of import. The AIA procedure is designed to ensure that before an LMO is imported into a country for the first time for intentional introduction into the environment, the Party of import is notified about the proposed import,

receives full information about the LMO and its intended use. The procedure gives an opportunity to assess the risks associated with that LMO and to decide whether or not to allow the import. A separate procedure has been established by the CPB for transboundary movement of LMOs-FFP, which includes communicating the decision through the BCH. Under this procedure, a Party must inform other Parties through the BCH within 15 days of its decision regarding domestic use of LMOs that may be subject to transboundary movement. While the AIA procedure is bilateral based on direct communication between Parties, the procedure for LMOs-FFP is essentially a multilateral information exchange mechanism centered on the BCH.

In addition, bilateral, regional, and multilateral agreements and arrangements for implementation of the CPB by Parties are also available on BCH.

2.3. Decisions and Declarations

Decisions and declarations by various countries are posted on the BCH. These include decisions on LMOs under AIA procedure, decisions for LMOs-FFP and other decisions and declarations that Parties have to make available to the BCH such as simplified procedure, transit, illegal or unintentional transboundary movement etc.

To facilitate easier understanding about results of queries involving decisions on LMOs, different icons have been used in the BCH. When highlighted these icons indicate the type of decisions used as for intentional release, direct use as food or feed or processing etc. (Table 2).

Table 2. Icons used for conveying information about decisions

Icon	Meaning
	Decision refers to an LMO for *Intentional introduction into the environment*.
	Approval of an LMO for *Direct use as food*.
	Decision refers to an LMO for *Direct use as feed*.
	Decision refers to an LMO for *Processing*.
	Decision refers to an LMO for *Confined Use*.
	Decision refers to an LMO for *Pharmaceuticals*.
	Approval of an LMO for *Transit*.

2.4. Risk Assessments

Risk assessment reports are mandatory for all decisions regarding the first import of LMOs for intentional introduction into the environment or regarding the domestic use of LMOs intended for direct use as food or feed, or for processing (LMOs-FFP) and should be consistent with Annex III of the CPB. It has been specified that the summary of the risk assessment of the effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health cannot be considered confidential information and should always be made available through the BCH when generated by regulatory processes.

2.5. Roster of Experts

The BCH provides access to a list of biosafety roster of experts, established to provide technical advice and other support, as appropriate and upon request, to developing country Parties and Parties with economies in transition, to conduct risk assessment, make informed decisions, develop national human resources and promote institutional strengthening, associated with the transboundary movements of LMOs.

The section on 'Roster of Experts' contains information on the experts as well as the guidelines for using the roster, including procedures for nominating experts and for updating information on the experts, the process of requesting and using experts from the roster, as well as information on the voluntary fund for the use of experts from the roster. Record of experts are maintained on the roster for a period of four years from the last update of their information, after which they are deleted unless renominated by Parties. "A Guide to the Roster of Biosafety Experts" is also available on BCH that serves as a quick reference to the roster of experts and the Voluntary Fund for the roster. It describes the nature, role and operational procedures for the roster.

2.6. National Reports

National reporting is a mandatory requirement under Article 33 of the CPB and these reports are submitted by Parties on four yearly basis. So far, four reports have been submitted by Parties i.e. interim report (2005), first national report (2007); second national report (2011) and third national report (2015). Copies of all the reports and their analysis are available on BCH.

3. LMOs, Genes or Organisms

Databases of LMOs, genes and organisms are maintained on BCH and are referred to as registries. These include description of LMOs, different types of genetic elements that can be used in the creation of new LMOs and characteristics of recipient or donor organism (non-LMOs) from which genes used for genetic transformation processes come from.

3.1. The LMO-Unique Identifiers Registry (LMO-UIds) provides summary information of all LMOs registered in the BCH including transformation events, genetic modifications, and the unique identification code (if available) for each record. Links to all decisions that refer to these organisms are provided at the bottom of each LMO record accessible through the registry.

The unique identification classifications serve as a key to access records in the BCH, such as the OECD's unique identifiers for transgenic plant lines for LMOs-FFP.

Box 3: OECD's 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 designate the transformation event; and
- 1 numerical digit for verification

Example: MON-00810-6 Monsanto's Yield Gard Maize

Unique identifier codes can be used to search BCH for information about specific LMOs

3.2. The Gene Registry, provides summary information on gene inserts and characteristics of the genetic modifications of LMOs; and

3.3. The Organism Registry, provides summary information on parental, recipient or donor organisms related to the LMOs registered in the BCH.

4. Capacity Building

The BCH also contains important information about biosafety capacity building and other assistance towards implementing CPB. Information about capacity building opportunities such as funding grants, scholarships and fellowships, technical assistance, training workshops, internships/ apprenticeships, study tours, partnerships, discussion forums and others is available on BCH. Similarly, information about capacity building projects, compendium of academically accredited biosafety courses, capacity-building needs and priorities is available on BCH.

5. BCH Virtual Library

The BCH provides access to different categories of information aimed to assist countries in capacity-building for implementation of the CPB through its virtual library. The two primary databases that make available biosafety information through the BCH Virtual Library are given below:

5.1. Biosafety Information Resource Centre (BIRC)

The Biosafety Information Resource Centre (BIRC), a sub-section of BCH contains electronic catalogues of biosafety-related publications and information resources for policymakers, educators, researchers, and the general public.

BIRC records may be registered by all BCH account-holders. The BIRC contains news services, e-mail list servers, online databases and search engines; reports and case studies; journals, newsletters and teaching material (manuals, toolkits and presentations). Its objective is to increase the accessibility and utilization of available biosafety information and resources for policymakers, educators, researchers, and the general public. Information from BIRC can be retrieved using various search options by clicking on the "Search the Biosafety Information Resource Centre (BIRC)".

Several search criteria boxes available on BIRC such as publication year, thematic area, type of record, language, date of record and keyword search. Using the search pages helps to learn about types of field and their operations.

5.2. Scientific Bibliographic Database on Biosafety (Bibliosafety)

The Scientific Bibliographic Database on Biosafety provides access to a bibliographic collection of scientific studies relevant to biosafety and risk assessment of biotechnology featuring records from CAB ABSTRACT database and maintained by the International Centre for Genetic Engineering and Biotechnology (ICGEB). This searchable database is updated

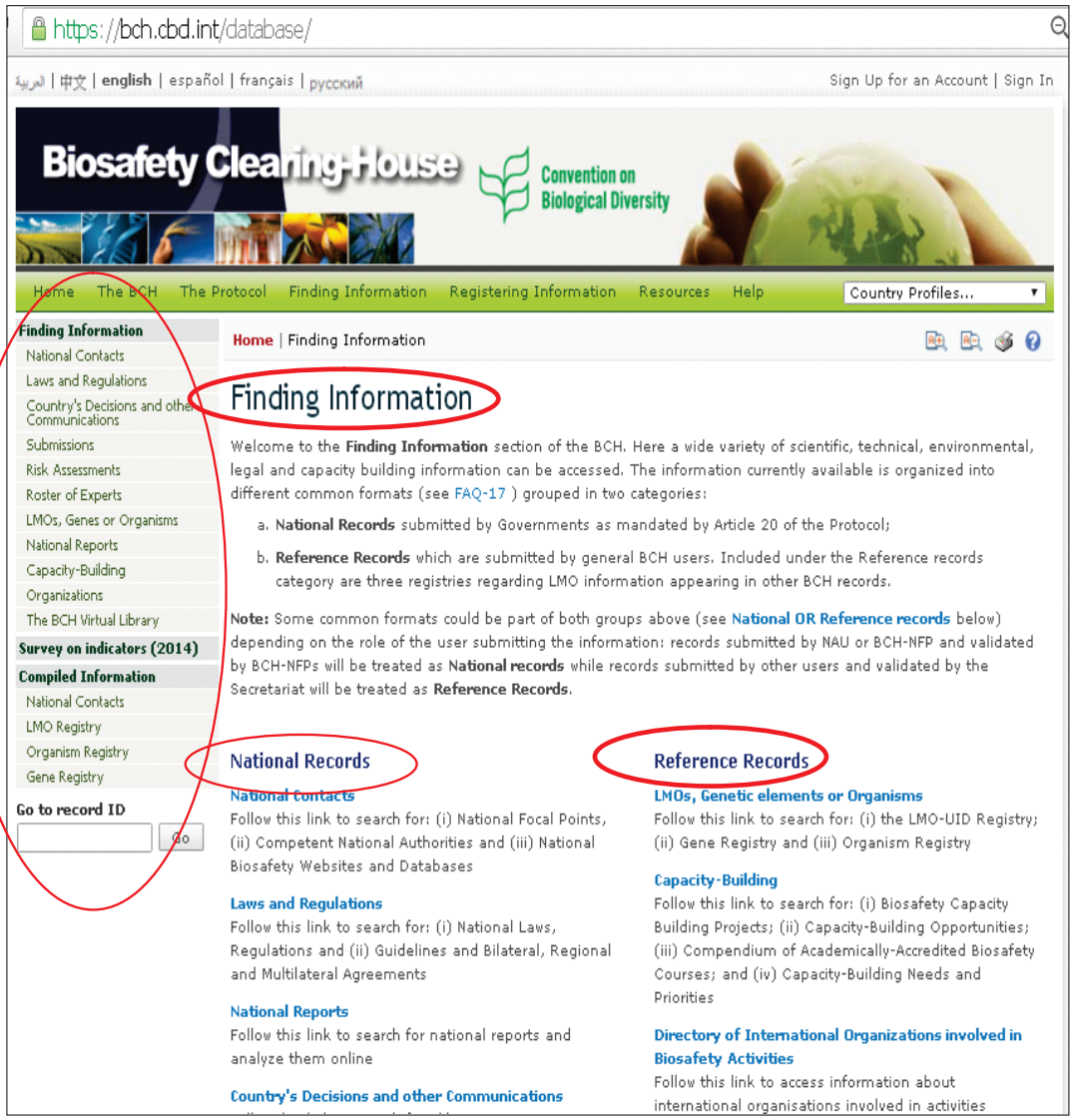
monthly and contains records of scientific articles (full reference + abstract) published in national and international scientific periodicals from 1990 onwards. Each record is vetted by ICGEB scientists for its contribution to the numerous scientific debates concerning Genetically Modified Organisms (GMOs).

Information about international organisations involved in activities relevant to implementation of the CPB with summaries of their activities and contact information is available at https://bch.cbd.int/database/organisations/.

6. Finding Information on the BCH Portal

Information registered in the BCH can be accessed through the **"FINDING INFORMATION"**, link on the navigation bar of the BCH website.

The 'Finding Information'section, allows access to all the above mentioned categories of information grouped under National and Reference Records.

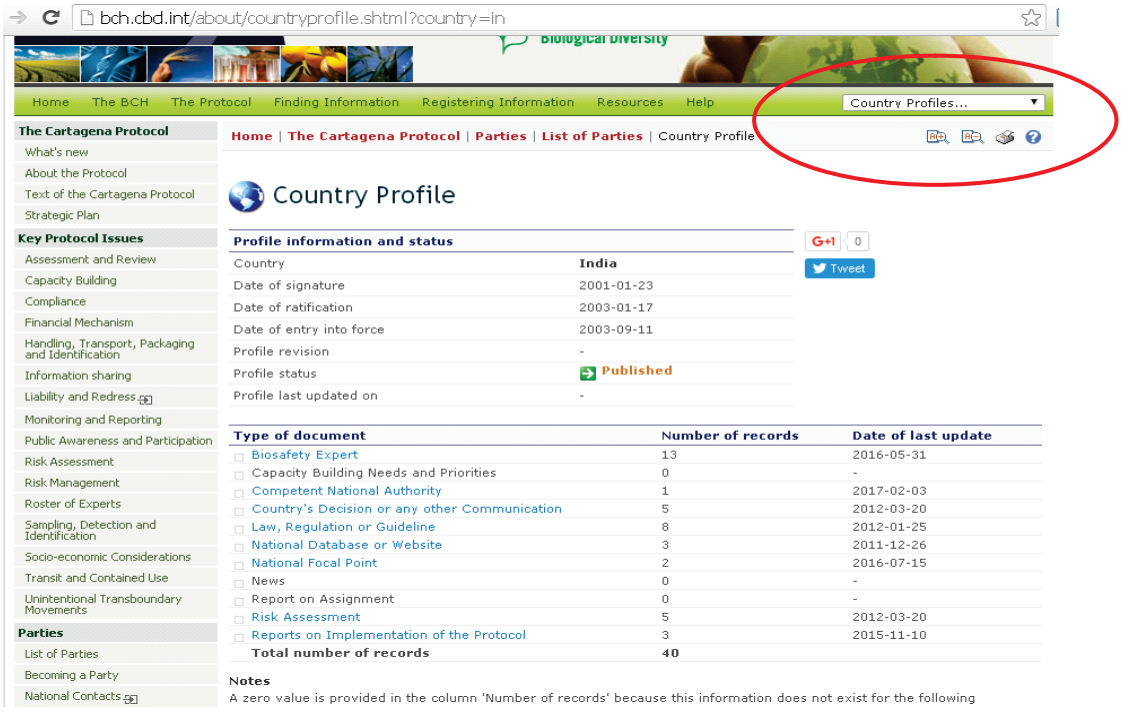


Specific information heads can be accessed from the left-hand menu, from the drop-down menu of the **Finding Information** link on the BCH navigation bar. After selecting the appropriate category, the search item screen will appear. From this search screen, a search for a record can be conducted using free text terms, or by using the special controlled vocabularies (i.e. consistent terms that have been translated into different languages and

are used to describe the content of the records). All search pages have a similar and consistent design.

For each category of information on BCH, search mechanisms have been provided for retrieval of information in a user friendly manner. The records of decisions, risk assessments, LMOs, donor and recipient organisms, and DNA sequences are cross-referenced in a way that facilitates data retrieval.

Every BCH page provides a quick search facility to obtain country profiles. A drop down menu is provided on the right hand side of the horizontal navigation bar which allows the user to select a country and display a summary of all the records entered in the BCH by that country.



7. Registered Users

In addition to general use of the website, BCH also has provision for registration of users. BCH registered users can log onto the Training Site of the BCH with their regular email address and password. This site provides access to training and capacity building material. In addition, interactive e-learning module is also available on BCH.

8. Conclusions

BCH is a repository of up-to-date information on LMOs and biosafety. As the official information-exchange mechanism under the Protocol, the BCH has been created with the intent of providing easy access to relevant and authenticated biosafety information. This information exchange mechanism can be used in many ways by different stakeholders to freely search and retrieve information through the BCH website. Governments use the BCH to make informed decisions regarding the importation or release of GMOs through analysis of relevant information, such as decisions on release and risk assessments. For industry, BCH provides easy access to vital information for their trade activities such as details of national contacts, relevant laws and regulations and decisions and declarations, especially relevant to imports and exports. The information about contact details of national authorities and decisions by various countries is also extremely useful for enforcement officials such as

customs and quarantine. Scientific and technical cooperation is fostered by allowing users to access or contribute information on capacity building activities and national priority needs. To conclude, BCH is an extremely important tool for easy and open access to key information and effective participation of multiple stakeholders.

9. References

- The Biosafety Clearing-House. https://bch.cbd.int/.
- Ministry of Environment, Forest and Climate Change and Biotech Consortium India Limited (2017) *Handbook on Biosafety Clearing House: An Information Sharing Platform.* http://in.biosafetyclearinghouse.net/phase2/publications.html.
- Galloway Mclean K (2005) Bridging the gap between researchers and policy-makers: international collaboration through the biosafety clearing-house. *Environmental Biosafety Research* 3:123-126.
- Secretariat of the Convention on Biological Diversity (2004) The Biosafety Clearing House of the Cartagena Protocol on Biosafety: A Guide to the BCH. Montreal: Secretariat of the Convention on Biological Diversity. https://www.cbd.int/doc/publications/bch-brochure-en.pdf.

Sampling Strategies: Bulk Material of Living Modified Organisms

9

V Celia Chalam, Shashi Bhalla, Chanda Priyadarshini and Ruchi Sharma
ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
Email: *celia.chalam@icar.gov.in; mailcelia@gmail.com*

1. Introduction

In case of bulk consignments, a proper sampling of the lot is to be done as per the norms for ensuring an effective processing of the material. The quantity of seed tested in the laboratory is minute compared with the size of the seed lot which it is intended to represent. It is important to note that no matter which analytical method is used, application of the correct sampling method is crucial for the result. The amount/ size of the sample drawn and the sampling methodology used are very crucial to enable detection of living modified organisms (LMOs)/ genetically modified organisms (GMOs) in the samples drawn. Sampling is the most crucial first step in any analytical process and is often the major source of error in the analysis of LMOs/GMOs. The overall objective of a good sampling plan is to provide a representative sample for the analysis and to minimize this error. It is imperative that the sampling step is performed as accurately as possible so that the sample collected is representative of the batch under investigation and to get the most accurate "true value". Obtaining representative samples deserves particular consideration since wrong sampling plan can greatly affect the reliability of the measured GMO/LMO levels.

The objective of sampling is to obtain a representative sample (from a seed lot) of a size suitable for tests, in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot. The sampling for LMOs/ GMOs is no different than any other characteristic in seed. To obtain uniform and accurate results, it is essential that the primary, composite and submitted samples be taken and prepared with care and in accordance with the prescribed methods. In order to use standardised methods for sampling of seed, the International Seed Testing Agency (ISTA) has developed a sampling guide ISTA Handbook on Seed Sampling, 2004 (Kruse, 2004). International Seed Testing Association (ISTA) Rules, 2018 gives the detailed sampling procedures for sampling of the seed for propagation and ISTA Chapter 2 of ISTA Rules, 2018 gives definitions of various sample types, including primary, composite, submitted and working samples, as well as guidelines

for obtaining seed lot samples that represent the properties of the seed lot . These definitions and guidelines also apply to GMO testing. Chapter 19 of ISTA Rules, 2018 deals with testing for seeds of GMOs.

The size of the submitted sample required for testing is small as compared to the size of the lot, therefore, care must be taken to ensure that the submitted sample represents the lot of the seed to be tested. Hence it is essential that the samples be prepared in accordance to ISTA rules to ensure that the small size sample should represent truly and in the same proportion all constituents of seed lot. No matter how accurately the laboratory work is done, the results only show the quality of the sample submitted for analysis; consequently, every effort must be made to ensure that the submitted sample accurately represents the composition of the seed lot. Like-wise, in reducing the sample in the laboratory, every effort must be made to obtain a working sample that is representative of the submitted sample.

2. Definitions (as per ISTA Rules, 2018)

2.1. Lot: A lot is a specified quantity of seed, physically identifiable

2.2. Sublot: A sublot is a portion of not less than 20% of the seed lot. Each container of a sublot must be marked with the identification of the seed lot.

2.3. Primary Sample: A primary sample is a portion taken from the seed lot during one single sampling action.

2.4. Composite Sample: The composite sample is formed by combining and mixing all the primary samples taken from the seed lot.

Subsample: A subsample is a portion of a sample obtained by reducing a sample.

2.5. Submitted Sample: A submitted sample is a sample that is to be submitted to the testing laboratory and may comprise either the whole of the composite sample or a subsample thereof. The submitted sample may be divided into subsamples packed in different material meeting conditions for specific tests.

2.6. Duplicate Sample: A duplicate sample is another sample obtained for submission from the same composite sample and marked 'Duplicate sample'.

2.7. Working Sample: The working sample is the whole of the submitted sample or a subsample thereof, on which one of the quality tests described in the ISTA Rules is made and must be at least the weight prescribed by the ISTA Rules for the particular test.

3. General Principle

A composite sample is obtained from the seed lot by taking primary samples from different positions in the whole seed lot and combining them. From this composite sample, subsamples are obtained by sample reduction procedures at one or more stages forming the submitted sample and finally the working samples for testing.

4. Procedure for Sampling the Seed Lot

4.1. Preparation of a Seed Lot and Conditions for Sampling

At the time of sampling, the seed lot must be as uniform as practicable. If the seed lot is found to be obviously heterogeneous, sampling must be refused or stopped. Seed may be sampled in containers or when it enters containers. The containers must be fit for purpose, i.e. must not damage the seed, and must be clean to avoid cross contamination. The containers must be labelled or marked before or just after sampling is completed. The seed lot must be so arranged that each part of the seed lot is conveniently accessible.

4.2. Minimum Sampling Intensity

For seed lots in containers holding up to and including 100 kg, the minimum sampling intensity is given in Table 1.

Table 1. Minimum sampling intensity for seed lots in containers holding up to and including 100 kg seed

No. of containers	Minimum number of primary samples to be drawn
1-4	3 primary samples from each container
5-8	2 primary samples from each container
9-15	1 primary samples from each container
16- 30	15 primary samples, one each from 15 different containers
31-59	20 primary samples, one each from 20 different containers
60 or more	30 primary samples, one each from 30 different containers

When sampling seed in containers holding more than 100 kg of seed, or from streams of seed entering containers, the sampling intensity according to Table 2 must be regarded as the minimum requirement.

Table 2. Minimum number of primary samples to be taken from seed lots in containers holding more than 100 kg of seed, or from seed streams

Seed lot size	Number of primary samples to be taken
Up to 500 kg	At least five primary samples
501–3 000 kg	One primary sample for each 300 kg, but not less than five
3 001–20 000 kg	One primary sample for each 500 kg, but not less than 10
20 001 kg and above	One primary sample for each 700 kg, but not less than 40

When sampling a seed lot of up to 15 containers, regardless of their size, the same number of primary samples must be taken from each container.

4.3. Collection of Primary Samples

When defining the number and/or the size of primary samples, the seed sampler needs to ensure (besides meeting the minimum sampling intensity) that the minimum amount of seed required for the requested test(s) is sent to the testing laboratory and enough seed remains available for obtaining duplicate samples, if requested.

Primary samples of approximately equal size must be taken from a seed lot, irrespective of where in the lot or container the primary sample is taken.

When the seed lot is in containers, the containers to be sampled must be selected at random or according to a systematic plan throughout the seed lot. Primary samples must be drawn from the top, middle and bottom of containers, but not necessarily from more than one position in any container, unless so specified in Tables 1 and 2. When the seed is in bulk or in large containers, the primary samples must be drawn from random positions. Containers must be opened or pierced for abstraction of primary samples. The sampled containers must then be closed or the contents transferred to new containers.

When seed is to be packed in special types of containers (e.g. small, not penetrable, or moisture-proof containers), it should be sampled, if possible, either before or during the filling of the containers.

The instruments being used must neither damage the seed nor select according to seed size, shape, density, chaffiness or any other quality trait. All sampling apparatus must be clean before use to prevent cross contaminations. Triers must be long enough so that the opening at the tip reaches at least half of the diameter of the container. When the container is

not accessible from opposite sides, the trier must be long enough to reach the opposite side. Sampling seed lots may be done by one of the methods listed below.

4.3.1. Automatic Sampling from a Seed Stream

Seed may be sampled by automatic sampling devices, provided that the instrument uniformly samples the cross section of the seed stream and the material entering the instrument does not bounce out again. It may be operated either under manual or automatic control. The intervals between taking primary samples should beconstant.

4.3.2. Manual Sampling from a Seed Stream

Seed streams may also be sampled by using manual instruments.

4.3.3. Sampling Stick

The sampling stick (e.g. stick trier, sleeve type trier (Fig. 1), spiral trier) consists of two parts, one of which fits loosely inside the other, but tightly enough so that seed or impurities do not slip between them. The outer part has a solid pointed end. Both parts have slots in their walls so that the cavity of the inner part can be opened and closed by moving the two parts against each other by either a twisting or a push-pull motion.

The sampling stick may be used horizontally, diagonally or vertically. The spiral trier has slots in a spiral arrangement for their subsequent opening from the tip to the handle and may only be used for seeds of a size smaller than *Triticum aestivum*.

However, when used vertically or diagonally downwards, the sampling stick must either have partitions dividing the instrument into a number of compartments or have slots in a spiral arrangement. The minimum inside diameter should be wide enough to allow the smooth and free flow of seed and contaminants into the sampling stick.

Fig 1. Sleeve type trier

When using the sampling stick, insert it in the closed position into the container, gently push it so that the point reaches the required position, open the sampling stick, agitate it slightly to allow it to fill completely, gently close and withdraw it and empty the primary sample into a container. Care should be exercised in closing the sampling stick so that seeds are not damaged.

4.3.4. Nobbe Trier

The Nobbe trier (dynamic spear) is a pointed tube with an opening near the pointed end. Seed passes through the tube and is collected in a container. The minimum internal diameter of the Nobbe trier (Fig. 2) should be wide enough to allow the smooth and free flow of seed and contaminants through the trier. When using the Nobbe trier, insert it at an angle of about 30° to the horizontal plane with the opening facing down, push the trier until it reaches the required position and revolve it through 180°. Withdraw it with decreasing speed from the container, gently agitating the trier to help maintain an even flow of seed, and collect the seed sample coming from the trier in a suitable container.

The name was given after the father of seed testing Fredrick Nobbe. This trier is made in different dimensions to suit various kinds of seeds. It has a pointed tube long enough to reach the centre of the bag with an oval slot near the pointed end. The length is very small. This is suitable for sampling seeds in bag.

Fig 2. Nobbe trier

4.3.5. Cargo Sampler

The cargo sampler (bulk sampler) consists of a special type of chamber that is fixed to a shaft. The lower part of the chamber is cone-shaped with a pointed end. To reach a greater depth, the shaft may be lengthened by screwing on successive extensions. There is a closing system in the chamber that may be a collar on the outside of the instrument, a wing connected to a door or a valve with a spring. Some cargo samplers can be closed

before they are drawn back from the sampling position; others cannot be closed, so that the filled chamber is open during withdrawal. For all species, the minimum inside diameter can be about 35 mm and the depth 75 mm. When using the cargo sampler, insert it in the closed position into the container, gently push it vertically into the seed so that the point reaches the required position, pull the cargo sampler back about 10 cm or turn it (depending on the closing system), agitate it slightly to allow it to fill completely, gently close if possible and withdraw it and empty the primary sample into a container. Care should be exercised in closing the cargo sampler, so that the seeds are not damaged.

4.3.6. Sampling by Hand

This method can be used for all species and may be the most suitable method for seed that may be damaged by the use of triers, seeds with wings, seeds with low moisture content, seed tapes and seed mats.

For hand sampling seed in containers, all positions inside the containers must be accessible. Containers with layers which are not accessible from the regular opening may have to be cut open, sampled and repackaged. Containers may also be partially or completely emptied during the sampling process to gain access to all positions in the containers. For sampling by hand, clean the hand and roll the sleeve up if necessary, insert the open hand into the container to the required position, close and withdraw the hand, taking great care that the fingers remain tightly closed about the seeds so none may escape, and empty the hand into a receiving pan.

4.4. Obtaining the Composite Sample

Where possible, the primary samples are compared with each other during sampling. The primary samples can only be combined to form the composite sample if they appear to be uniform. If not, the sampling procedure must be stopped. When primary samples are collected directly into one container, the content of this container may be regarded as the composite sample only if it appears uniform. If not, it must not be used for obtaining a submitted sample.

4.5. Obtaining the Submitted Sample

The submitted sample must be obtained by reducing the composite sample to an appropriate size by one of the methods referred to in 4.2.

Duplicate samples requested not later than at the time of sampling, must be prepared in the same way as the submitted sample.

4.5.1. Weight of Submitted Sample

ISTA rules provide minimum weight of submitted samples for various agricultural, horticultural, and tree seeds are given in Table 3.

Table 3. Minimum weight of submitted sample

Crop species	Minimum weight of submitted samples
Avena sativa, Triticum aestivum, Hordeum vulgare, Zea mays, Phaseolus spp., *Pisum sativum, Cicer arietinum Secale cereale, Leucaena leucocephala, Glycine soja, Gossypium* spp., *Helianthus annuus, Arachis hypogea, Lupinus* spp., *Pinus pinea, Fagus sylvatica, Vicia* spp., *Vigna* spp., *Cucurbita* spp., *Cajanus cajan, Dolichos lablab*	1000 g
Prunus avium, Sorghum vulgare	900 g
Beta vulgaris, Prunus serotina	500 g
Oryza sativa, Calopogonium mucunoides	400 g
Sorghum sudanense, Trifolium subterraneum, Spinacia oleracea	250 g
Sinapsis alba	200 g
Capsicum spp., *Cucumis melo, Cucumis sativus, Pennisetum typhoides, Solanum melongena, Linum usitatissimum*	150 g
Brassica spp., *Pinus caribaea*	100 g
Allium cepa, Stylosanthes spp.	80 g
Sesamum indicum, Allium porum	70 g
Cuminum cyminum, Trifolium alexandrium	60 g
Malus spp., *Medicago lupilina, Medicago sativa, Meliolotus* spp., *Rosa* spp., *Trifolium pratense, Cichorium intybus, Allium fistulosum*	50 g
Brassica chinensis, Cichorium endivia, Picea abies, Brassica nigra	40 g
Lactuca sativa, Daucus carota, Ulmus spp.	30 g
Nicotiana tabacum, Apium graveolens, Solanum lycopersicum	25 g

Source: ISTA (2018)

4.6. Dispatch of the Submitted Sample

The submitted sample must be marked with the same identification as the seed lot. For an Orange International Seed Lot Certificate, the sample must be sealed. The additional information required as well as the name of any chemical treatment applied must be provided.

Submitted samples must be packed so as to prevent damage during transit. Submitted samples should be packed in breathable containers.

Subsamples for moisture testing, and samples from seed lots which have been dried to low moisture content, must be packed in moisture-proof containers which contain as little air as possible.

Submitted samples must be dispatched to the seed testing laboratory without delay.

5. Procedure for Obtaining the Submitted and Working Sample

5.1. Minimum Size of Working Sample

The working sample is a sub-sample of the submitted sample prepared in the laboratory according to ISTA methods. It shall contain a minimum of 3000 seeds (Lübeck, 2014). The size of the working sample for GMO detection depends on given threshold requirements, the method capability and the degree of required statistical confidence, and can be determined using appropriate statistical tools. The sample submitted to the laboratory must therefore be at least the size of the working sample, but more realistically larger than the working sample. The size of the sample must be consistent with the performance of the analytical method in terms of limit of detection in order to allow the detection of even one GM seed in the sample. For quantitative methods, the size of the sample must be consistent with the limit of quantification to allow the quantification of even one GM seed (http://www.seedtest.org/upload/cms/user/ISTA-TCOMs-June15-1600-GMO-Dollardetal.pdf).

The recommended laboratory (working) sample size by European commission (2014) in JRC Technical Report, Guidelines for sample preparation procedures in GMO analysis is given in Table 4 and Table 5.

Table 4. Recommended laboratory (working) sample size according to the type of matrix

Products	Recommended laboratory sample size
Seeds	Mass equivalent of 3000 kernels (see table 5 for mass equivalent of 1000 kernels)
Commodity grains	Mass equivalent of 10000 grains (see table 5 for mass equivalent of 1000 kernels)
First transformation products (semolina, flour, grits, oilcake etc.)	From 100 g to 1 kg
Liquids	500 ml
Doughy and viscous products	500 g
End products (e.g. packed rice noodles)	From 100 g to 1 kg

Source: European commission (2014)

Table 5. Data concerning the mean mass of 1000 kernels for different plant species

Plant species	Mean mass of 1000 kernels (in g)
Barley	37
Linseed	6
Maize	285
Millet	23
Oat	32
Rapeseed	4
Rice	27
Rye	30
Soybean	200
Sugar beet	11
Sunflower	100
Tomato	4
Wheat	37

Source: European commission (2014)

5.2. Sample Reduction Methods

If the seed sample needs to be reduced to a size equal to or greater than the size prescribed, the seed sample must first be thoroughly mixed. The submitted/working sample must then be obtained either by repeated halving or by abstracting and subsequently combining small random portions. The apparatus and methods for sample reduction are given below:

5.2.1. Mechanical Divider Method

This method is suitable for all kinds of seeds except some very chaffy seeds. The apparatus divides a sample passed through it into two or more approximately equal parts. The submitted sample can be mixed by passing it through the divider, recombining the parts and passing the whole sample through a second time, and similarly, a third time if necessary. The sample is reduced by passing the seed through repeatedly and removing parts on each occasion. This process of reduction is continued until a working sample of approximately, but not less than, the required size is obtained. The apparatus used include conical divider, soil divider, centrifugal divider, rotary divider and variable sample divider. One, two or more of these methods may be used in one sample reduction procedure. After obtaining a working sample the remainder must be re-mixed before a second working sample is obtained.

5.2.2. Modified Halving Method

The apparatus comprises a tray into which fits a grid of equal-sized cubical cells, open at the top and every alternate one having no bottom. After preliminary mixing, the seed is poured evenly over the grid. When the grid is lifted, approximately half the sample remains on the tray. The submitted sample is successively halved in this way until a working sample, of approximately but not less than the required size, is obtained.

5.2.3. Spoon Method

The spoon method is recommended for sample reduction for seed health testing. For other tests it is restricted to species with seeds smaller than *Triticum* spp., to the genera *Arachis*, *Glycine* and *Phaseolus*, and to tree genera *Abies*, *Cedrus* and *Pseudotsuga*. A tray, a spatula and a spoon with a straight edge are required. After preliminary mixing, pour the seed evenly over the tray; do not shake the tray thereafter. With the spoon in one hand, the spatula in the other, and using both, remove small portions of seed from not less than five random places. Sufficient portions of seed are taken to constitute a subsample of the required size.

5.2.4. The Hand Halving Method

This method is restricted to the genera of chaffy seeds viz., *Agrimonia, Andropogon,*

Anthoxanthum, Arrhenatherum, Astrebla, Beckmannia, Bouteloua, Brachiaria, Briza, Cenchrus, Chloris, Dichanthium, Digitaria, Echinochloa, Ehrharta, Elymus, Eragrostis, Gomphrena, Gossypium (linted seed only), *Melinis, Oryza, Pennisetum* (non *glaucum*), *Psathyrostachys, Scabiosa, Sorghastrum, Stylosanthes* (non *guianensis*), *Trisetum*; the genera of easily damaged fragile seeds viz., *Arachis, Glycine* and *Phaseolus* and the genera and species of tree and shrub seeds viz., *Acer, Aesculus, Ailanthus, Castanea, Cedrela, Corylus, Fagus, Fraxinus, Juglans, Liriodendron, Pinus cembra, Pinus pinea, Platanus, Populus, Quercus, Salix, Tectona* and *Ulmus*.

The hand halving method (Fig. 3) can also be used with the species where all other dividing methods are extremely difficult or impossible to use. For all other species it can be used only to obtain working samples in the laboratory for seed health tests.

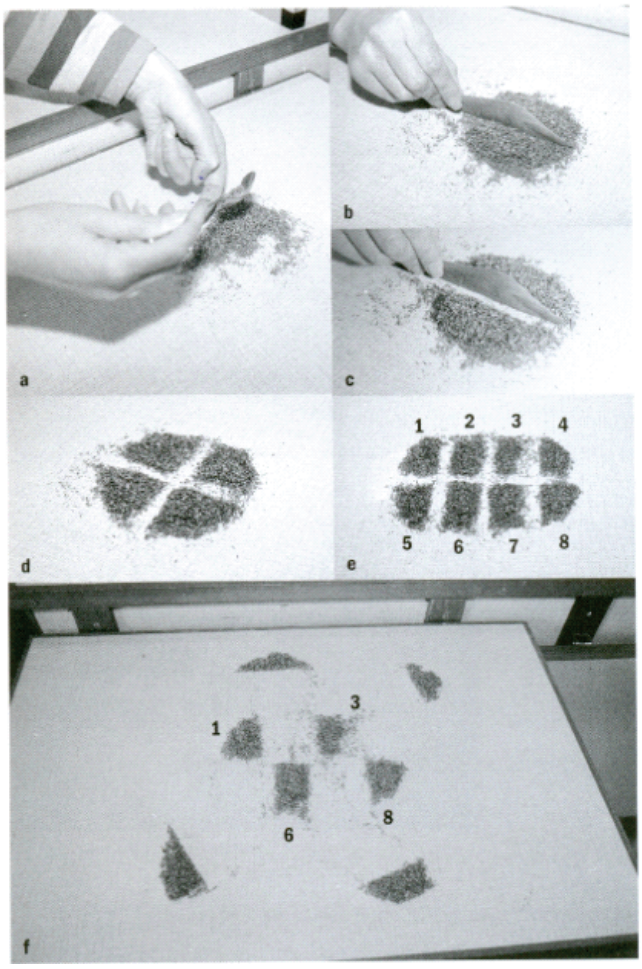


Fig. 3. Hand-halving method

For applying the hand halving method, pour the sample evenly onto a smooth clean surface, thoroughly mix the seed into a mound with a flat-edged spatula, divide the mound into half and halve each half again - giving four portions - and halve each portion again - giving eight portions, arrange the portions in two rows of four, combine and retain alternate portions: e.g. combine the first and third portions in the first row with the second and fourth in the second row, remove the remaining four portions. Repeat the procedure using the retained portions until obtaining the required sample size.

6. Storage of Submitted Samples

Every effort must be made to start testing a submitted sample on the day of receipt. Storage of orthodox seeds, when necessary, should be in a cool, well-ventilated room. Non-orthodox (i.e. recalcitrant or intermediate) seeds should be tested as soon as possible after obtaining the submitted sample from the composite sample without any storage. Handling of the submitted sample and, if necessary, storage should be done under species specific optimum conditions.

7. Cleanliness in Sample Preparation

Carry over of material from one sample to another takes on an even greater significance during sample preparation prior to analysis. Due to the sensitivity seen with many methods for detection of GMOs/LMOs, care must be taken to avoid transferring materials to subsequent samples. Whole seeds, dust and residual matter must be removed from all equipment. Grinders should be cleaned through vacuuming of dust, washing with soap and water or solvents, or a combination of appropriate cleaning methods for the specific grinder in use. Sample dividers and mixers must also be thoroughly cleaned. Analysts should verify the equipment cleaning process is appropriate to prevent cross contamination. Many of the analytical techniques practiced for detection of GMOs/LMOs can detect levels lower than 0.1%. Physical separation of sample preparation operations from analytical operations is also highly recommended to avoid contamination of sample extracts.

8. Perspectives

Seed sampling is the first substantial part of seed quality control, starting from drawing the primary samples from the seed lot, up to obtaining the representative working sample of a suitable size for the appropriate test. The test results are expected to reflect the average quality of the seed lot, therefore accuracy in sampling is of fundamental importance. Incorrect sampling may lead to misleading test results, discarding seed lots of high quality, or to the approval of seed lots of low quality, which may reduce crop yield or even result in complete

failure (Arne Wold, 1986). The sampling plans must be used in conjunction with an established procedure for collection of primary samples from the bulk sample and preparation of the composite sample to ensure that the composite sample is representative of the seed lot.

In India, quarantine processing of bulk consignments of grain/ pulses etc. for consumption and seed/ planting material for sowing are undertaken by the Directorate of Plant Protection, Quarantine and Storage (DPPQS) under the Ministry of Agriculture and Farmers' Welfare (MoA&FW) through its 53 Plant Quarantine Stations operational in different parts of the country. The bulk material for sowing/ planting purposes are authorized only through five Plant Quarantine Stations located at New Delhi, Mumbai, Chennai, Kolkata and Amritsar. The DPPQS has developed and notified Standard Operating Procedures for Export inspection and phytosanitary certification of plants/ plant products and other regulated articles (http://plantquarantineindia.nic.in/pqispub/pdffiles/SOP-Export%20Inspection.pdf), which includes detailed sampling procedures and the same sampling procedures are followed for imported seed and other planting material. The sampling procedures for seed for propagation are in accordance with the International Rules for Seed Testing of ISTA. The samples collected for quarantine processing would be used for GMO/ LMO detection also.

9. References

- European commission (2014) *European Commission JRC Technical Report: Guidelines for Sample Preparation Procedures in GMO Analysis*. Prepared by the ENGL ad hoc working group on "sample preparation procedures", Luxembourg. 38 p.
- http://plantquarantineindia.nic.in/pqispub/pdffiles/SOP-Export%20Inspection.pdf *Standard Operating Procedures for Export Inspection and Phytosanitary Certification of plants/ Plant Products and Other Regulated Articles.* Retrieved June 14, 2017.
- ISTA (2018) *International Rules for Seed Testing*. The International Seed Testing Association, Switzerland.
- Kruse M (2004) *ISTA Handbook on Seed Sampling*, 2nd ed.; International Seed Testing Association: Bassersdorf, Switzerland.
- Lübeck Mette (2018) Detection of genetically modified plants - methods to sample and analyse GMO content in plants and plant products. https://www.researchgate.net/publication/237644324_Detection_of_genetically_modified_plants_-methods_to_sample_and_analyse_GMO_content_in_plants_and_plant_products.

Detection of Living Modified Organisms: Immunodiagnostics

10

V Celia Chalam, Shashi Bhalla, SC Dubey, Chanda Priyadarshini, Aradhika Tripathi, Ruchi Sharma and AK Maurya
ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
Email: *celia.chalam@icar.gov.in; mailcelia@gmail.com*

1. Introduction

Advances in biotechnology have enabled the development and production of Genetically Modified Organisms (GMOs)/ Living modified organisms (LMOs) with properties such as tolerance to herbicides, resistance to insects and the addition of nutrition values. Transformation of plants is done by inserting DNA into a single cell, which is then regrown into a complete organism, the plant. DNA is the blueprint of each cell that is transcribed into the messenger RNA (mRNA), which is then translated into a protein.

2. Need for Detecting LMOs

Concerns have been raised globally as to whether these GMOs/LMOs are safe for human beings, animals and to the environment. These concerns have led to demand to regulate and perhaps label seed, feed and food products to inform the consumer whether the products being imported or marketed are made of genetically modified (GM) seed or plants. A processed food manufacturer needs to demonstrate that a food product does or does not contain GMOs such as starlink (Bt) protein in corn or the Roundup (RR) transgene in corn or soybean. An organic farmer needs to ensure that the seed or planting material being used is free from GMOs. A researcher needs to profile and identify a newly developed GMO. Similarly, a seed company needs to certify that it is producing and marketing pure inbred or hybrid seed, or GM seed. The quarantine stations need to test for GMOs in commodities under trade and also germplasm and research material under exchange.

The deliberate or inadvertent mixing of GM seed with non-GM seed lots carries the risk of adversely affecting international seed trade. Already several such cases, causing economic losses to seed companies, have come to light. In addition to exasperating discussions over permissible threshold of GMOs in non-GM seed lots, the establishment of reliable, efficient and cost-effective techniques for detection, identification and quantification of GMOs in non-GM seed lots present significant challenges.

The **Article 18** of Cartagena Protocol on Biosafety (CPB) requires safe handling, transport, packaging and identification of LMOs that are subject to transboundary movement. The parties are required to have specific measures in place to ensure that these may include specifying documentation requirements, strengthening enforcement systems and facilities for sampling, detection and identification of LMOs. The CPB also requires Parties to take sound measures in their domestic regulation to address the issue of unintentional transboundary movement **(Article 17)** and illegal transboundary movement **(Article 25)** of LMOs.

Detection and identification of LMOs is a cross-cutting issue and relevant to a number of biosafety-related issues, such as risk assessment and management, detection of unauthorized or illegal LMOs, detection of unintentional introductions into the environment, and liability and redress. Thus, the capacity to detect and identify LMOs is arguably one of the main pillars for the effective implementation of the provisions of the CPB and domestic biosafety legislations.

3. Detection of LMOs

All testing methodologies currently available for LMOs, detect either the novel DNA or the novel protein. These detection methods can be divided into four categories. *Screening* methods have the broadest application, as they are suitable for detecting multiple LMO/GMO traits. *Trait-specific* methods detect a specific novel protein whilst *construct-specific* methods detect a specific DNA construct used to introduce the novel trait. Finally, *event-specific* methods provide unambiguous identification of a specific transformation event. In many situations, a test will be required that not only detects the presence of LMOs in commodities but also measures the amount of LMO present in the sample. This additional requirement for quantification will impact on the most appropriate testing method for that application (Griffiths *et al*., 2007).

The LMOs can be detected by identifying DNA or RNA or protein. A majority of methods focus on detecting DNA, while only a few for detecting proteins or RNA. DNA can be purified and multiplied in billions of copies in just a few hours with Polymerase Chain Reaction (PCR) technique. Multiplication of RNA and proteins is a more complicated and slow process. DNA is a very stable molecule, while RNA is less stable. The stability of protein varies and depends on the type of protein. There is normally a linear correlation between quantity of GMO and DNA if the genetically modified DNA is nuclear, but not if it is extranuclear. However, there is usually no such correlation between the quantity of GMO/ LMO and protein/ RNA.

For the detection of LMOs/GMOs at the level of DNA, PCR-based methods are mainly used, whereas for protein-based detection, immunoassays such as enzyme-linked immunosorbent assay (ELISA) and lateral flow strips methods are predominantly used. RNA-based methods rely on specific binding between the RNA molecule and a synthetic RNA or DNA.

There are already a broad range of PCR-based methods in routine use. Methods using PCR technology seem to be very powerful for detecting LMOs/GMOs but are difficult in their application and expensive in the traditional seed testing context.

4. Detection of LMOs by Immunoassays

Immunoassay is based on the specific binding between an antigen and an antibody. A substance having high molecular weight (>10,000 daltons) when introduced into an animal, causes the formation of specific proteins (the immunoglobulins) in the blood, which are commonly called antibodies. The causative substance is called antigen and the blood serum containing antibodies is called antiserum. Positive reaction confirms the presence of the target protein (Khetarpal and Kumar, 1996).

Earlier immunoassays based on immunoprecipitation, immunodiffusion, and latex agglutination were very popular. Now a days ELISA is the most widely used method for detection of specific proteins as they are much more sensitive than diffusion and agglutination methods, use less antibody and can be employed for simultaneous handling of a large number of samples in routine testing.

Among the immunoassays most commonly used are the classical ELISA test (plate-based) and the Lateral flow Strip Method (membrane-based).

4.1. Enzyme-linked Immunosorbent Assay

The development of ELISA technique began in the field of diagnostic medicine when enzyme labeled antibodies were used for detection of antigens in tissues. Soon after, it was demonstrated that enzyme-labeling could yield quantitative assays with a sensitivity comparable to radioimmunoassays. ELISA has been the most widely used technique for the detection of viruses.

Of the various forms of ELISA test, the double-antibody sandwich form (DAS-ELISA) is most commonly used (Fig. 1). In this method, virus specific antibodies are adsorbed to solid surface (microtitre plates) and to this the sample to be tested is added which is followed by the addition of enzyme labeled specific antibody. The labeled antibodies bind to the antigen which is already bound to the coating antibodies. Finally, the substrate of the enzyme used is

added. The substrate is hydrolyzed and the development of colour in the end product is measured as absorbance values in a multiscan spectrophotometer. The colour change in the substrate is proportional to the amount of enzymes present which in turn is proportional to antigen concentration. Several "indirect" ELISA methods have also been developed in order to use one universal antibody conjugate.

The repetitive nature of the operations involved in ELISA make this technique well suited to automation and simultaneous testing of a large number of samples. The advantage of ELISA is in addition to qualitative diagnosis, that it can also quantify the targeted protein, provided a standard curve is employed.

Thus, the availability of antibodies with the desired affinity and specificity is the most important factor for setting-up immunoassay systems. This technique is ideal for qualitative as well as quantitative detection of many types of proteins.

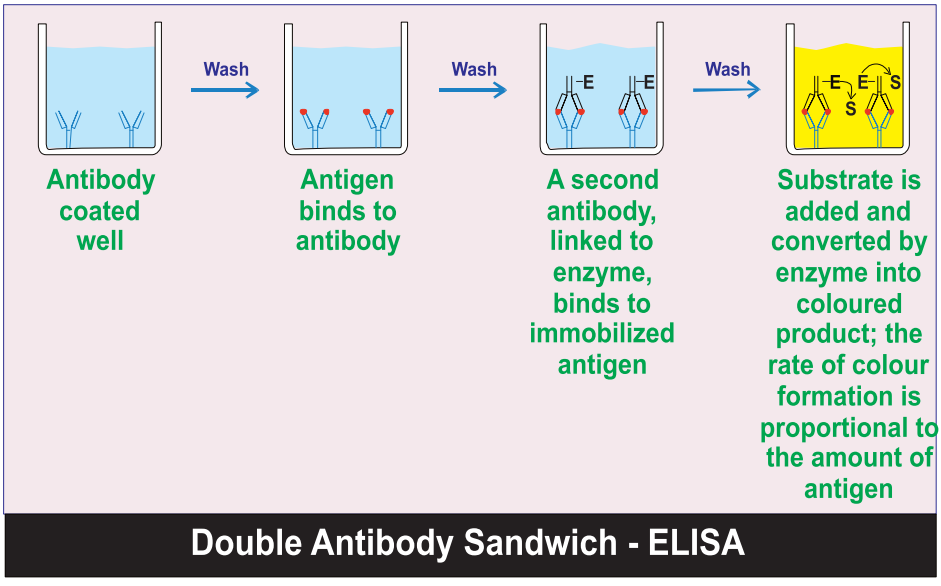


Fig. 1. Double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA)

4.2. Lateral Flow Strip Method

Lateral flow strip method is a variation of ELISA, but the antibodies are immobilised onto a test strip in specific zones. The test is provided in kit form and does not require any major equipment. Lateral flow strips are suitable for field or on-site use, with minimal training required. Sample preparation simply involves crushing the sample and mixing it with the extraction solution provided in the kit.

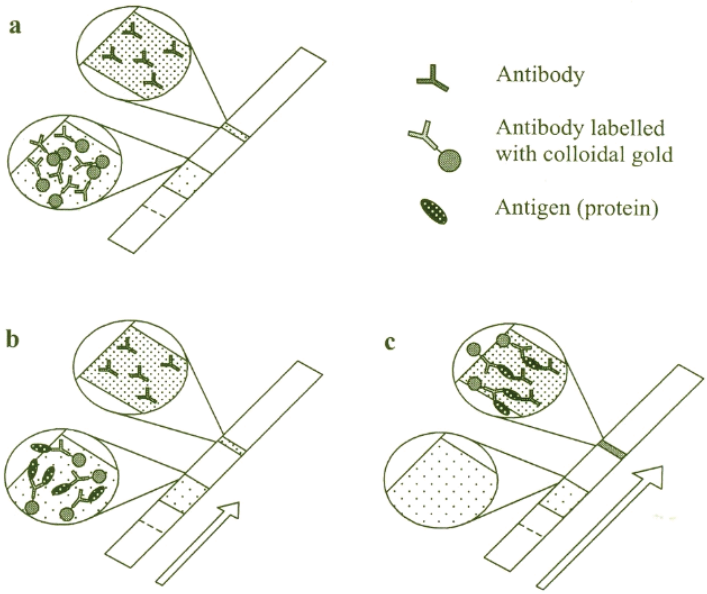


Fig. 2. Lateral flow strip method (Source: Griffiths *et al*., 2007)

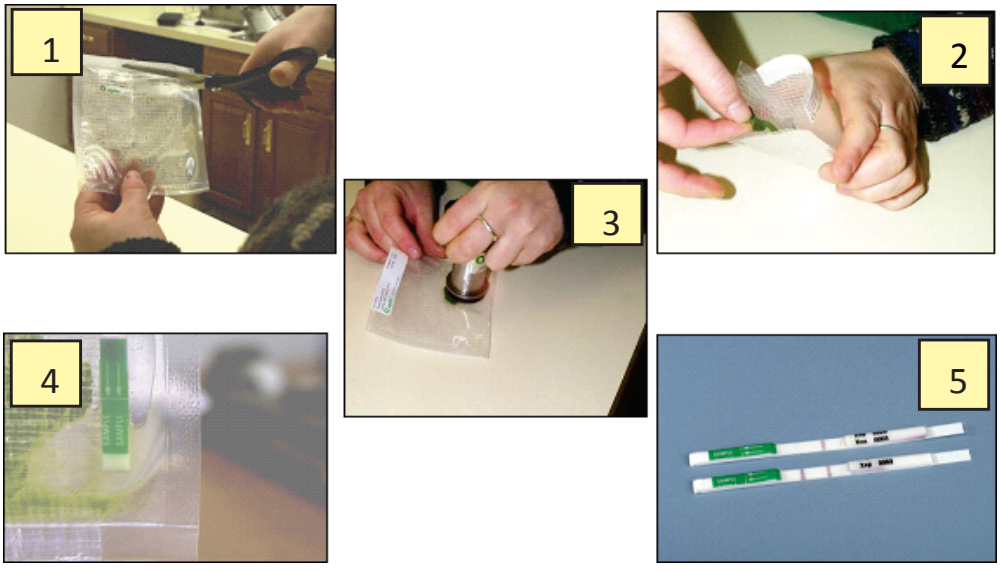
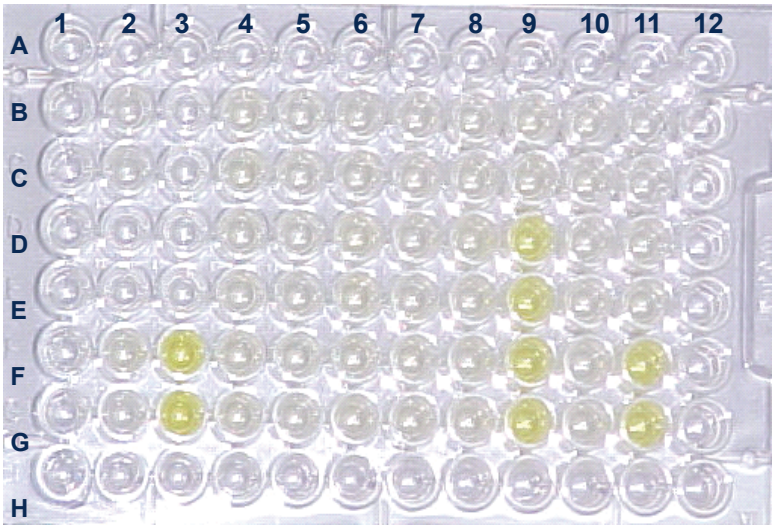


Fig. 3. Steps involved in performing lateral flow strip method (Source: www.agdia.com)

The lateral flow test strip is dipped into the prepared sample in extraction solution and the sample migrates up the strip by capillary action. As it moves up the strip, the sample passes through a zone of reagent that contains antibodies, usually labeled with colloidal gold. This labeled antibody binds to the GM protein, if present in the sample. The antibody-

protein complex then continues to move up the strip until it reaches a second zone of antibodies, which in this case are immobilised onto the test strip. The complex concentrates into this immobilised antibody zone where the gold becomes visible as a red band. The test strip also contains an immobilised control zone that binds a control complex that is present in the extraction solution and also produces a visible line. If there is no target GM protein present only a single line will form at the control zone. A result is called positive when both the control line and the line indicating presence of target GM protein change colour. These tests generally provide qualitative or semi-quantitative results using antibodies and colour reagents incorporated into a flow strip (Fig. 2 and Fig. 3).

A number of organisations viz., Association of Analytical Chemists, (AOAC), the US; Codex Alimentarius Commission, Italy; EU Joint Research Centre (JRC), Italy; International Seed Testing Association (ISTA), Switzerland; USDA/ Grain Inspection, Packers and Stockyards Administration (GIPSA), the US etc. are involved in development and validation of the diagnostic kits by conducting ring trials/ proficiency tests. GMO analysis laboratories should participate in proficiency tests organized by independent bodies, to regularly, test and demonstrate that their analyses are reliable and accurate. For industry, control authorities and others purchasing GMO analyses, it is highly recommendable to require from the laboratories that they are accredited, that they participate in proficiency tests, and that the laboratories also make public know how they perform these tests. Preferably, the analyses methods should be of international standards, to avoid disputes between parties using different methods.



3FG, 9DE& 9FG: Bt cotton; 11FG: Positive control of Bt cotton;
2FG: Negative control; 2BC: Buffer control; Other wells: Non-Bt cotton

Fig. 4. DAS-ELISA of Bt cotton seed samples

Several groups in the world including Central Institute for Cotton Research (CICR), Nagpur in India, have developed ELISA and immunostrip systems. Also, a number of ELISA and lateral flow/ immunostrip test kits are commercially available and the details are given in Table 1. At ICAR-NBPGR, commercial kits viz., Bt-express kit and Bt Quant ELISA kit of CICR, Nagpur and ELISA-based diagnostic kits of Monsanto and Envirologix Inc., USA were tested by detecting transgenes in cotton seed samples (Fig. 4).

Table 1. Diagnostic kits available for testing LMOs

Gene	Test format
AAD-12 (cotton Enlist™ trait resistant to 2,4-D herbicide)	Immunostrip[a]
Amylase protein in Enogen corn derived from transformation Event 3272 (corn)	ELISA[d] LOD 1% (one positive grain in 100 grains)
Barnase (mustard/canola)	ELISA[b]
Barstar (mustard/canola)	ELISA[b]
*cry*1A & *cry*2A (cotton)	LFD[d]
*cry*1A & *cry*3B (corn)	ELISA[d]
*cry*1A, Event 603 (CP4 EPSPS), *cry*3Bb, *cry*1F, T25-PAT/pat, *cry*34, m*cry*3A (MIR604), *cry*2A, and/or Vip3A (corn)	LFD[d] up to nine different lateral flow membrane strips (dipsticks) custom-assembled into comb format LOD 0.25% (one kernel in 400) to 1% (one kernel in 100)
*cry*1A, Event 603 (CP4 EPSPS), *cry*3Bb, *cry*1F, T25-PAT/pat, *cry*34, modified *cry*3A (MIR604), Vip3A, or *cry*2A (corn)	LFD[d] LOD 0.25% (one kernel in 400) to 1% (one kernel in 100)
*cry*1A, *cry*2A, CP4 EPSPS, DMO, and Vip3A (cotton)	LFD[d]
*cry*1Ab (corn, cotton, soybean)	ELISA[b] corn, LFT[b] corn;ELISA[c] cotton; ELISA[e]

	qualitative; ELISA[f] soybean, LOD 0.15-2.0%; LFD[f] corn, bulk seeds, LOD 0.9%
*cry*1Ab and *cry*1A.105 (MON810, MON89034, and Bt11 corn)	LFD[d*] LOD0.8% (one positive kernel in 125)
*cry*1Ab/-1Ac	Qualitative DAS-ELISA[a]; Immunostrip[a];ELISA[b] cotton; ELISA[d] corn and cotton; ELISA[e] qualitative and quantitative
*cry*1Ab-specific, *cry* 2Ae, 2mEPSPS, and PAT/*bar (cotton)*	LFD[d]
*cry*1Ab/CP4 (corn)	LFT[b] corn
*cry*1Ab/PAT (corn)	LFD[f] corn, bulk grain, LOD 0.9 %
*cry*1Ac (corn, cotton, soybean)	ELISA[b] cotton, soybean; LFT[b] cotton, soybean; ELISA[c] cotton; LFD[d] cotton, bulk seed, LOD0.25% (one positive seed in 400); ELISA[e] qualitative & quantitative; LFD[f] corn, bulk grain, LOD 0.9 % and soybean, bulk grain, 0.5%
*cry*1Ac, *cry*1Ab (cotton)	DS[c], cotton
*cry*1Ac &*cry*2A (cotton)	Dual trait DAS-ELISA[a]; ELISA[b]; LFT[b]; ELISA[d]
*cry*1Ac, *cry*2A, and CP4 EPSPS (cotton)	LFT[b], LFD[d]
*cry*1A, *cry*2A, CP4 EPSPS, DMO, and Vip3A (cotton)	LFD[d]
*cry*1Ac, *cry*2A, CP4 EPSPS, and PAT/bar (cotton)	LFD[d]
*cry*1Ac, *cry*2A, 2mEPSPS, and PAT/*bar* (cotton)	LFD[d]
*cry*1Ac, *cry*1F and/or CP4 EPSPS (cotton)	LFD[d]

*cry*1Ac, *cry*1F, CP4 EPSPS, and Vip3A (cotton)	LFD[d]
*cry*1C (cotton)	ELISA[b]; ELISA[d] LOD 0.2ppb
*cry*1EC (cotton)	ELISA[b]
*cry*1F (corn and cotton)	Quantitative DAS-ELISA[a] corn, Herculex®; Qualitative DAS-ELISA[a] corn, Herculex® I and Herculex® XTRA; Immunostrip[a] corn, LOD 0.5%, (one positive seed in 200); ELISA[b] corn, cotton; LFT[b] corn, cotton; ELISA[c] cotton; ELISA[d] LOD 0.17% Herculex I corn or WideStrike cotton; LFD[d*] LOD 0.5% (one positive kernel in 200); ELISA[e] qualitative & quantitative; LFD[f*] corn, bulk grain, LOD 0.9%
*cry*1F, *cry*1Ac & CP4 EPSPS (corn)	Multi-analyte Immunostrip[a]
*cry*1F &*cry*34Ab1 (HERCULEX® XTRA, HERCULEX® RW, HERCULEX® I and SmartStax® seed corn)	Multi-analyte DAS-ELISA[a]; ELISA[d]
*cry*1F &*cry*34Ab1(HERCULEX® XTRA, seed corn)	Dual-trait Immunostrip[a]
*cry*2A (corn and cotton)	Qualitative DAS-ELISA[a] corn; ELISA[b] corn, cotton; LFT[b] corn, cotton; ELISA[d] cotton, LOD 0.52 ppb; LFD[d] cotton, LOD0.25% (one in 400); LFD[d*] cornLOD0.9% (~8 in 800)
*cry*2A, *cry*3Bb, *cry*1F, and *cry*34 (corn)	LFD[d]
*cry*2Ab (corn event MON89034; cotton)	Immunostrip[a]; ELISA[c] cotton;LFD[d*] corn, bulk grain, LOD1% (one in 100); ELISA[e] qualitative & quantitative; LFD[f]
cry2Ab &*cry*1Ab/1Ac (cotton Bollgard II)	Immunostrip[a]

*cry*2Ab, *cry*1Ac & CP4 EPSPS (cotton BGII & RR)	Multi-analyte Immunostrip[a]
*cry*2Ab &*cry*3Bb1(corn events MON 89034 and/or MON 88017)	Dual-trait DAS-ELISA[a]
*Cry*2Ae (cotton)	LFD[d] cotton, bulk seed, LOD 0.5% (one positive seed in 200)
*cry*3A (potato NewLeaf®)	DAS-ELISA[a]
*cry*3Bb (corn) 0.125 %	ELISA[b]; LFT[b];LFD[f] corn, bulk grain, LOD
*cry*3Bb1 (corn, YieldGard® Rootworm, YieldGard® Plus, YieldGard VT Rootworm/RR2® and YieldGard VT Triple®)	DAS-ELISA[a]; Immunostrip[a] LOD 1% (one positive seed/leaf in 100 corn seeds/leaves); ELISA[d] LOD 0.1% YieldGard corn
*cry*34Ab1 (corn)	Quantitative and Qualitative DAS-ELISA[a]; Immunostrip[a]; ELISA[d]; LFD[d*] corn, bulk grain, LOD0.5% (one in 200); LFD[f] corn, bulk grain, LOD 0.125%
CP4 EPSPS (alfalfa, canola, corn, cotton, soybean, sugarbeet)	DAS-ELISA[a], bulk composite testing, LOD 0.1% for all crops; Immunostrip[a], LOD0.1% (one in 1000 seeds); ELISA[b] corn, cotton, soybean; LFT[b] corn, cotton, soybean; ELISA[d], corn, bulk grain, LOD 0.1%; ELISA[d] soybean Roundup Ready, LOD 0.1%; ELISA[d] soybean RR2Y, LOD 0.2%; LFD[d*] alfalfa, LOD 0.1% (weight/weight ratio); LFD[d*]canola, bulk grain, LOD 0.1% (one positive kernel in 1000); LFD[d*] corn, bulk grain, LOD 0.5% (one positive kernel in 200); ELISA[d] qualitative & quantitative; ELISA[f] soybean, 0.3-2.5%; LFD[f] alfalfa, bulk grain, LOD 0.167%; LFD[f] canola, LOD 0.1%; LFD[f] corn, LOD 0.125%; LFD[f] soybean and sugarbeet, LOD 0.1%
CP4 EPSPS (RR) &*cry*1Ab/1Ac	Immunostrip[a]

CP4 EPSPS, *cry*1A (*cry*1Ab, *cry*1Ac, *cry*1A.105), *cry*3Bb (corn)	LFD[f] corn, bulk grain,LOD 0.5-0.9 %
CP4 EPSPS, *cry*1A.105, *cry*3Bb, *cry*1F, PAT, *cry*34Ab1, VIP3A (corn)	LFD[f] semi-quantitative, low-LOD, corn comb
CP4 EPSPS, *cry*3Bb, *cry* 1A.105, *cry* 1F, *cry* 34Ab1, PAT, and/or VIP3A (corn)	LFD[f] bulk grain, semi-quantitative, corn comb
CP4 EPSPS, PAT, *cry* 1A (*cry* 1Ab, *cry* 1Ac, *cry* 1A.105), *cry*3Bb, *cry* 1F, *cry* 34Ab1, VIP3A	LFD[f]
CP4 EPSPS, PAT (soybean)	LFD[f]
CspB (corn)	Qualitative DAS-ELISA[a]; Immunostrip[a] LOD 1% in a minimum sample size of 300 seeds; LFD[d*] bulk grain, LOD1%; LFD [f] corn, bulk seeds, LOD 0.9%
DMO (cotton)	LFD[d] LOD 0.5% (one in 200)
DMO (soybean)	Immunostrip[a] soybean, LOD 0.25% (one in 400); LFD[d*] LOD 0.25% (one in 400)
e*cry*3.1Ab (corn event 5307, Agrisure Duracade®)	DAS-ELISA[a];Immunostrip[a] LOD 1% (one in 100); ELISA[b] corn, bulk seed; ELISA [d] LOD 0.25%; LFD[d] LOD 1%; LFD[f] corn, bulk grain, LOD 0.25 %
GUS (cotton)	ELISA[c] cotton
HPPD (cotton, soybean)	ELISA[b] cotton, soybean; LFT[b] cotton, soybean
m*cry*3A (corn)	Immunostrip[a];ELISA[b] bulk seed; LFT[b]; ELISA[d]; LFD[d*] bulk grain, LOD 1% (one in 100)
NPT II	TAS-ELISA[a]
NPT II (cotton Roundup Ready®)	Immunostrip[a]
PAT (canola, corn, rice, sugarbeet)	LFD[f] bulk grain, LOD 0.9 % in corn and

	sugarbeet, 2% in canola, 0.05 % in LLRice62 and 2 % in LLRice61
PAT/*bar* (canola, corn, cotton, mustard, rice, soybean)	ELISA[b] canola, corn, cotton, mustard, soybean; LFT[b] canola, mustard; ELISA[d] LOD 0.1%; LFD[d] cotton, bulk seed, LOD 0.25% (one in 400); LFD[d] rice, bulk grain, LOD 1.33% Event LL601 (one positive seed in 75), 0.02% Event LL62 rice (1:5000)
PAT/*pat* (canola, corn, soybean)	ELISA[b] corn, cotton, soybean; LFT[b] corn; ELISA[d] LOD 0.2%; LFD[d*] canola, bulk seeds, LOD 0.25% (one in 400); LFD[d*] corn and soybean, bulk grain, LOD 0.5% (one positive kernel in 200)
PAT/*pat* and CP4 EPSPS (soybean)	LFD[d] LOD 0.25% (one in 400) to 0.5% (one in 200)
PAT/*pat,* CP4 EPSPS, DMO (MON87708) (soybean, Liberty Link and Roundup Ready)	LFD[d] LOD 0.1% (one in 1000) to 0.5% (one in 200)
PMI	LFD[f]
Vip3A (corn)	DAS-ELISA[a]; Immunostrip[a] LOD 0.25% (one positive seed in 500); ELISA[b] corn , bulk seed also; LFT[b] corn; ELISA[d];LFD[f] corn, bulk grain, LOD 0.33%
Vip3A (cotton)	ELISA[b]; LFT[b]; LFD[d]* LOD 0.5% (one in 200)
2mEPSPS (cotton)	ELISA[d];LFD[d] LOD 0.25% (one in 400)

DS: Dip stick; ELISA: Enzyme-linked Immunosorbent Assay; DAS-ELISA: Double Antibody Sandwich-Enzyme-linked Immunosorbent Assay; LOD: Limit of Detection; LFD: Lateral flow device; LFT: Lateral flow test

[a]Agdia Inc., USA; [b]Amar Immunodiagnostics, Hyderabad, India; [c]ICAR-Central Institute for Cotton Research, Nagpur, India; [d]EnviroLogix Inc., USA; [e]Krishgen Biosystems, India; [f] Romer Labs, USA

*reagents none required, wáter extraction

5. Pros and Cons of Using Immunoassays

There are certain inherent disadvantages of ELISA-based techniques as they require significant lead-time for method development, have high up-front costs for assay development, and cannot discriminate between different transgenic events that express similar protein characteristics. Also GM proteins might be produced only during certain developmental stages or in certain plant parts and such GMOs are unlikely to be detected with ELISA. Some GMOs do not express a detectable level of the target protein, whereas others are designed that do not produce a novel protein at all. Besides, protein detection methods are best suited to raw or partially processed samples, as many food-processing steps, including cooking, will cause the proteins to unfold or 'denature'. To overcome this limitation, some antibodies have been produced that recognize the protein in its denatured state and so are suited for testing cooked food. Protein detection methods are generally less sensitive than DNA detection methods. Protein detection relies on the amount of protein produced or 'expressed' by the novel DNA construct and also on whether that protein is expressed in the part of the plant being tested (Chalam and Khetarpal, 2007).

Nevertheless, these limitations do not reduce the importance of ELISA-based assays and it has its own place in the GMO detection toolbox along with the PCR-based techniques. On a routine basis, ELISA is incomparable when it comes to the traits that produce substantial quantities of GMO proteins. Besides, ELISA is found to be a technique of choice when presence of GMO is to be tested in bulk samples using appropriate sampling procedures. ELISAs are easy to use, robust and cheaper than DNA detection methods.

Also the lateral flow strip formats are ideally suited for on-site testing and require minimum sample preparation. Detection can be carried out by unskilled persons on-site, producing an answer in minutes. Protein detection methods are highly suitable for monitoring specific GM traits during handling of raw products, provided the protein is expressed in the part of the plant being tested. Thus, despite certain disadvantages, immunoassays have immense potential for reliable detection of GMOs in the fields itself, do not depend on the equipment and supply of costly enzymes and chemicals for every test and permit to handle a relatively large number of samples. The detection kits, available commercially, can be adopted without any special skills, and the cost of testing per sample is also comparatively very low. Also, the tests that detect multiple novel proteins using a single lateral flow strip were developed.

The choice of a technique for detecting LMOs/GMOs would, however, depend upon the purpose, the developmental stages of the host to be tested, and the threshold of detection limit.

6. References

- Chalam VC and Khetarpal RK (2007) Detection of GMOs by Immunoassays. In: *Cartagena Protocol on Biosafety: Decisions to Diagnostics* Randhawa GJ, Bhalla S, Chalam VC and Sharma SK (eds). National Bureau of Plant Genetic Resources, New India. 128-136 pp.

- Crowther JR (1995) *ELISA Theory and Practice*. Humana Press, Totowa, NJ, USA. 223 p.

- Griffiths K, Partis L, Croan D, Wang N and Emslie KR (2007) Review of technologies for detecting genetically modified materials in commodities and food. Australian Government Analytical Laboratories. http://www.daff.gov.au/_data/assets/pdf_file/0008/196982/AGAL_report.pdf. Retrieved March 13, 2003.

- Khetarpal RK and Kumar CA (1996) ELISA Methodology. In : *Molecular Methods in Plant Pathology*. Singh RP and Singh US (eds). CRC - Lewis Publishers, Boca Raton, USA. 329-342 pp.

Detection of Living Modified Organisms: DNA-based Techniques

11

Gurinderjit Randhawa and Monika Singh
ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
Email: *gurinder.randhawa@icar.gov.in*

1. Introduction

Genetically modified (GM) crops with desired traits are developed by introducing "gene or genetic construct of interest" employing recombinant DNA techniques. "Transgene(s)" being introduced in GM crops, either confers a new trait to the plant or enhance an already existing trait. By the end of 2017, area under GM cultivation has reached to a total of 189.8 million hectares covering 24 countries. Soybean, maize, cotton and canola are major globally commercialized GM crops, with herbicide tolerance and insect resistance as predominant traits (http://www.isaaa.org/).

In India, *Bt* cotton is growing in an area of 11.4 million hectares. Several other GM events have been imported for research purposes or are under field trials in the country. With increased number of GM events with diversified traits, GM detection has become more challenging, which gives a way forward to the GM detection research. Qualitative testing methodologies can be used for screening and detection of specific GM events, while quantitative testing can be used for estimating the GM content. GM detection methods can target either the transgenic DNA or the novel recombinant protein(s) expressed in GM crop. DNA-based methods are being employed because of higher specificity, sensitivity and wider applicability for processed/unprocessed samples.

Based on the target amplification, DNA-based detection methods can be categorized as: (i) Target amplification methods, as in polymerase chain reaction (PCR); and (ii) Signal amplification methods, as in real-time PCR. In the recent past, DNA-based GM diagnostics based on different approaches, such as GMO matrix, loop-mediated isothermal amplification (LAMP) and PCR-based multi-target system have also been developed.

GM detection performed in a step-wise manner is summarized in Fig. 1.

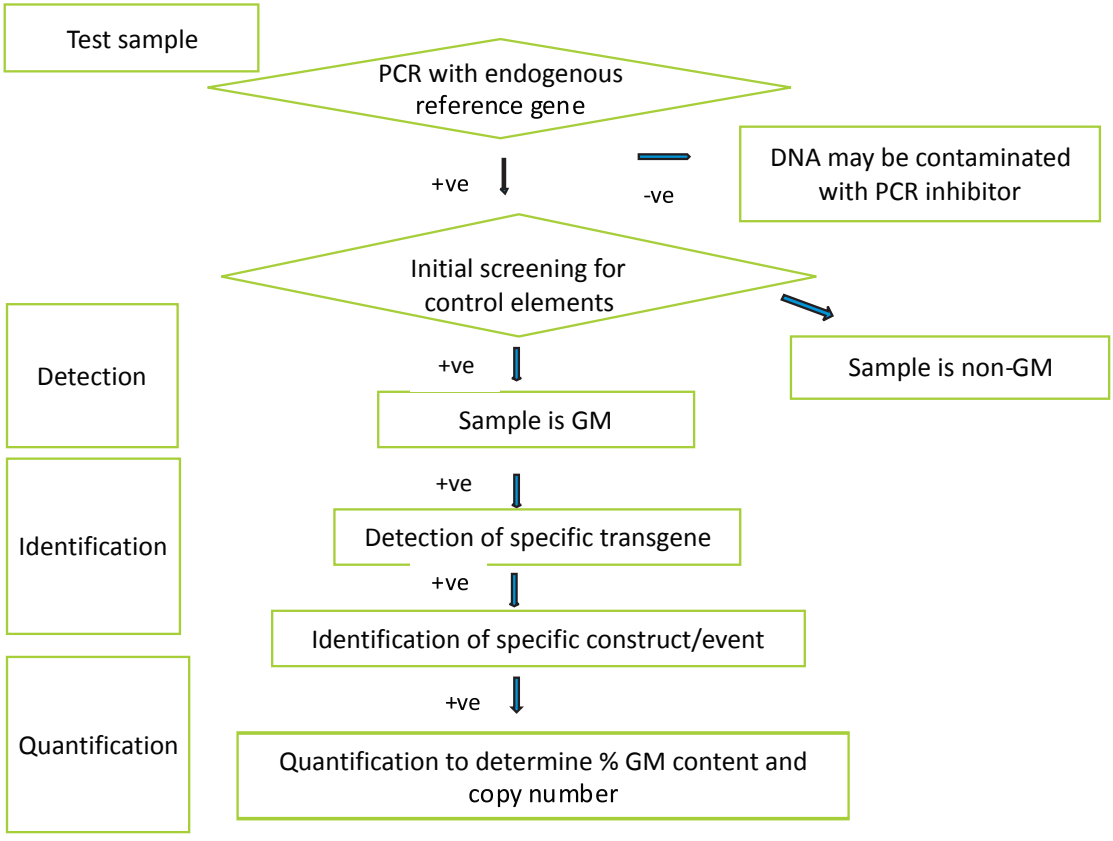


Fig.1. Sequential work flow for GM detection

2. Different DNA-based Techniques for Detection of GM Crops

2.1. Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) involves targeted amplification of transgenic elements using specific primers. Transgenic construct comprise of: (i) a promoter, which drives the expression of inserted gene; (ii) the inserted transgene conferring a specific trait to the host plant; (iii) a marker gene for selection of transformants; and (iv) the terminator, which acts as a stop signal. PCR-based GM detection methods are categorized on the basis of the level of specificity (Fig. 2): (i) screening methods targeting most commonly employed genetic elements, including promoters and terminators, for screening of GM crops; (ii) gene-specific methods targeting specific transgenes used in the transformation of plants; (iii) construct-specific methods targeting the junction between two DNA elements, for instance, a region of the insert spanning junction between promoter and transgene; and (iv) event-specific methods targeting the junction at integration locus between the recipient genome and inserted DNA.

Multiplex PCR, a variant of conventional PCR, involves simultaneous amplification of multiple target sequences in a test sample. Multiplex PCR simultaneously detecting commonly employed marker genes were also reported which could be employed to check the GM status irrespective of GM crop or trait (Randhawa *et al*., 2009). Multiplex PCR assays in decaplex format for detection of commercialized *Bt* cotton events have been developed (Randhawa *et al*., 2010; Chhabra *et al*., 2014)

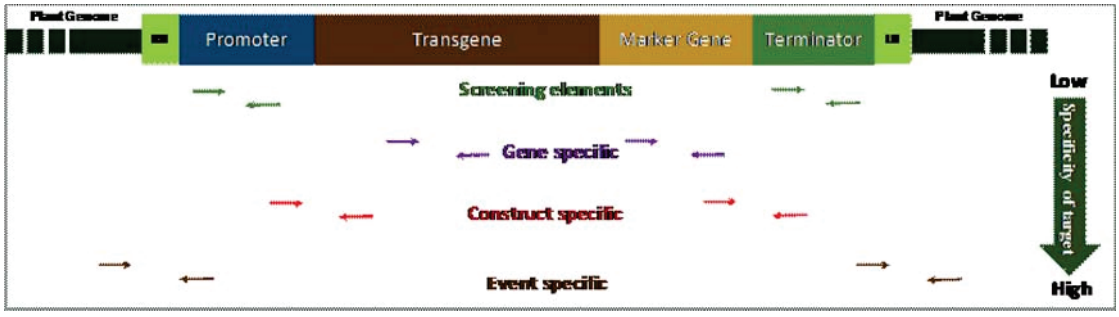


Fig. 2. Targets for DNA-based GM detection. The primer positions are depicted by arrows

Source: Randhawa *et al*., 2016

2.2. Real-time PCR

Real-time PCR allows monitoring of product, by measuring fluorescence signal produced during the progress of reaction. Fluorescent signals can be detected using DNA binding fluorescent dyes, e.g., SYBR Green® or more specific fluorescent probes (Fig. 3). A number of real-time PCR assays have been developed for detection and quantification of different GM events in several crops.

TaqMan® real-time PCR-based ready-to-use multi-target analytical system for detection of GMOs has also been developed, which reduces number of steps and minimizes handling error and chances of cross-contamination. The developed system consists of a 96 well pre-spotted plate with lyophilized primers and probes for a total of 47 assays in duplicate allowing simultaneous detection of GM events from corn, eggplant, rice, soybean and cotton; in particular, the system combined 21 events- specific assays, 6 taxon-specific assays, 5 construct regions and 15 element-specific assays, LOD ranged from 0.01-1%, depending upon the target (Randhawa *et al*., 2014).

2.3. Matrix-based Approach

GM detection laboratories initially undertake PCR-based preliminary screenings followed by more specific identification and quantification, if required. As testing directly for each

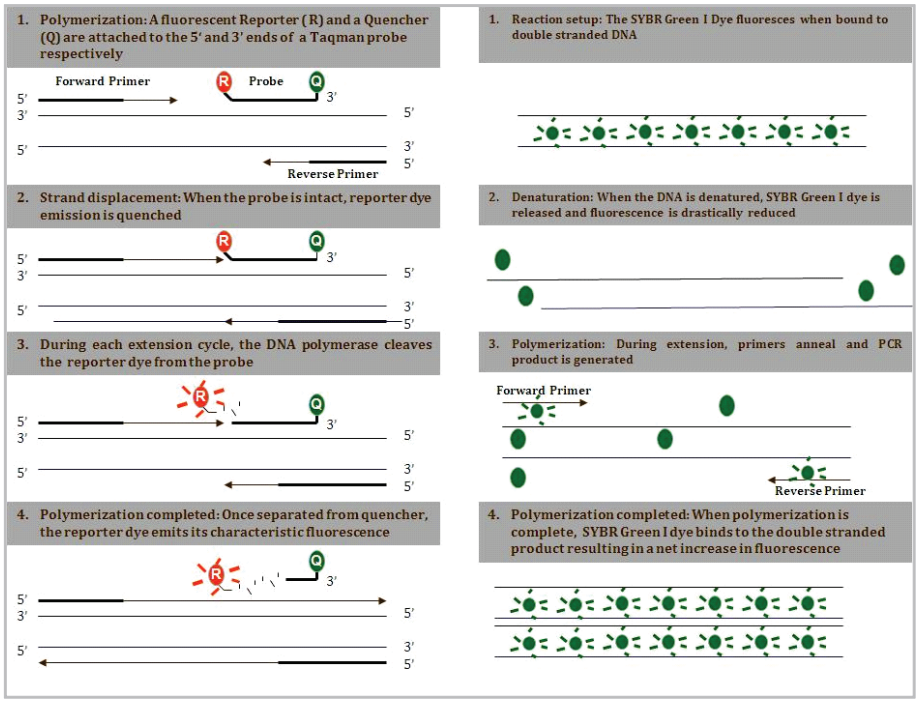


Fig. 3. Different chemistries used in real-time PCR technology

Source: http://technologyinscience.blogspot.in/2013/05/taqman-assay-vs-sybr-green-assay.html

target is extremely labor-intensive and costly, use of initial screening targeting commonly employed transgenic elements can facilitate time- and cost-efficient discrimination of GM and non-GM samples (zel *et al*., 2012). "Matrix-based approach" is an efficient and cost-effective strategy to check authorized GM events (Van den *et al*., 2010). GMO matrix is represented in the form of a table, in which each row represents a GM event, where as columns represent the analytical test methods or *vice-versa*. The GMO seek algorithm freely available on the webpage (http://kt.ijs.si/software/GMOtrack/) is used for development of GMO matrix.

GMO screening matrix was developed to check authorized GM events in India, for detection of 141 GM events of 21 crops based on the information of 106 genetic elements (Randhawa *et al*., 2014). Out of 106 genetic elements, 10 most frequently present targets were identified to screen these events. Matrix approach facilitates efficient, rapid and cost-effective screening by eliminating the need for development of specific testing methodologies for each individual GM event.

The concept of crop-specific GM matrix combined with multiplex PCR was also developed for GMO screening of globally approved GM events of cotton and maize, which

could be utilized by low resource GM testing laboratories in the country (Singh *et al*., 2016).

2.4. Loop-mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acids amplification technique, in which amplification and detection of target genes are completed in a single step at a constant temperature (Notomi *et al*., 2000). LAMP is characterized by the use of four different primers, which recognize six distinct regions on the target. An inner primer pair containing sequences of sense and antisense strands of the target DNA initiates LAMP reaction, which proceeds at a constant temperature, followed by strand displacement DNA synthesis primed by an outer primer pair. Addition of "loop" primers increases the specificity and time-efficiency of LAMP assays (Nagamine *et al*., 2002).

LAMP products show ladder-like pattern on agarose gel or can be real-time monitored using turbidimetry or by measuring fluorescence using real-time LAMP. The amplicons can alternatively be visualized after completion of the LAMP reactions using nucleic acid staining or fluorescent dyes such as SYBR® Green 1.

LAMP assays have been employed in GM diagnostics in the recent years, due to their time-efficiency, robustness and ease-of-use. LAMP-based visual and real-time screening assays targeting commonly used promoters, viz., *p35S, pFMV* and marker genes, *viz., aadA, nptII* and *uidA* have been developed (Randhawa *et al*., 2013). Visual and real-time LAMP assays targeting three commonly employed transgenes, namely, *cry1Ac, cry2Ab2* and *cp4-epesps* were reported (Singh *et al*., 2015). Event-specific visual and real-time LAMP assays for detection of two major commercialized *Bt* cotton events, viz., MON531 and MON15985 were developed (Randhawa *et al*., 2015). The flexibility of these LAMP assays facilitates their applicability for reliable GM detection on-site, if combined with a fast DNA extraction method. This approach would be useful for GMO screening by Plant quarantine/ customs authorities to check the consignments at ports of entry or by the field inspectors or farmers in the fields.

Different strategies being employed for GM detection have been summarized in Fig. 4.

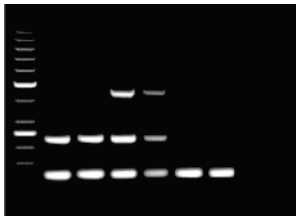
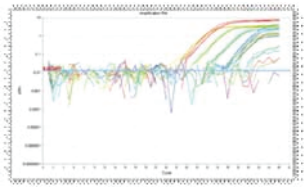
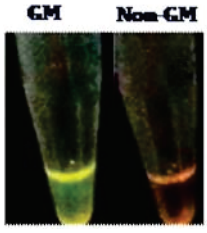
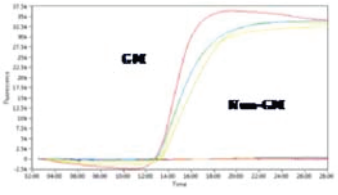
Multiplex PCR Multiple targets can be simultaneously detected using gel electrophoretic analysis	
Real-time PCR Targets can be detected and quantified using fluorometric analysis	
Visual LAMP Targets can be detected visually by addition of fluorescent dyes such as SYBR® Green I in the form of colorimetric change; may facilitate cost-efficient and rapid GMO testing	
Real-time LAMP Targets can be detected real-time using fluorometric analysis in the form of amplification and annealing curves; may facilitate on-site GMO testing when coupled with fast DNA extraction kit	
Multi-target TaqMan real-time PCR Plate Primers and probes lyophilized on a pre-spotted plate simultaneously detecting multiple targets in a run, facilitating rapid GMO testing.	

Fig. 4. DNA-based GMO detection techniques being employed in India

3. Conclusions

With increase in number of GM events and diversification of traits, cost-effective GM diagnostics could facilitate effective risk assessment and management of GM crops and for their post-release monitoring, to ensure public confidence and solve legal disputes. PCR and real-time PCR-based assays are being widely employed for GM detection and quantification due to their specificity, sensitivity and robustness. Strategies/technologies based on GMO matrix, LAMP, real-time PCR-based multi-target system are gaining popularity in GM detection due to wider applicability or cost-efficiency.

4. References

- Chhabra R, Randhawa GJ, Bhoge RK and Singh M (2014) Qualitative and quantitative PCR-based detection methods for authorized genetically modified cotton events in India. *Journal of AOAC International* 97: 1299-1309.
- http://www.isaaa.org/
- Nagamine K, Hase T and Notomi T (2002) Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and Cellular Probes* 16: 223-229.
- Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N and Hase T (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* 28: e63.
- Randhawa GJ, Chhabra R and Singh M (2009) Multiplex PCR-based simultaneous amplification of selectable marker and reporter genes for the screening of genetically modified crops. *Journal of Agricultural and Food Chemistry* 57: 5167-5172.
- Randhawa GJ, Chhabra R and Singh M (2010) Decaplex and real-time PCR-based detection of MON531 and MON15985 *Bt* cotton events. *Journal of Agricultural and Food Chemistry* 58: 9875-9881.
- Randhawa GJ, Chhabra R, Bhoge RK and Singh M (2015) Visual and real-time event-specific loop-mediated isothermal amplification based detection assays for *Bt* cotton events MON531 and MON15985. *Journal of AOAC International* 98: 1207-1214.

- Randhawa GJ, Morisset D, Singh M and el J (2014) GMO matrix: A cost-effective approach for screening for unauthorized genetically modified events in India. *Food Control* 38: 124-129.
- Randhawa GJ, Singh M, Morisset D, Sood P and el (2013) Loop-mediated isothermal amplification: Rapid visual and real-time methods for detection of genetically modified crops. *Journal of Agricultural and Food Chemistry* 61: 11338-11346.
- Randhawa GJ, Singh M, Sood P and Bhoge RK (2014) Multitarget real-time PCR-based system: Monitoring for unauthorized genetically modified events in India. *Journal of Agricultural and Food Chemistry* 62: 7118-7130.
- Singh M, Bhoge RK and Randhawa GJ (2016) Crop-specific GMO matrix-multiplex PCR: A cost-efficient screening strategy for genetically modified maize and cotton events approved globally. *Food Control* 70: 271-280.
- Singh M, Randhawa GJ, Sood P and Bhoge RK (2015) Loop-mediated isothermal amplification targeting insect resistant and herbicide tolerant transgenes: Monitoring for GM contamination in supply chain. *Food Control* 51: 283-292.
- Zel J, Milavec M, Morisset D, Plan D, Van den EG and Gruden K (2012) How to reliably test for GMOs? Springer, New York, 1st ed.

GLOSSARY

Advance Informed Agreement (AIA)	Principle or procedure (under the Cartagena Protocol on Biosafety) whereby the international exchange of resources or products that could have adverse effects on the environment should not proceed without the informed agreement of, or contrary to the decision of, the competent authority in the recipient country.
Adventitious presence of genetically Modified (GM) material	Detection of unintentional presence of GM crops that have not been approved in any country.
Biodiversity	The variability among living organisms from all sources, including inter alia, terrestrial, marine and other ecosystem and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.
Biosafety	Policies and procedures to manage the risks to human and animal health and safety, and to the conservation of the environment, as a result of activities with genetically modified organisms.
Biosafety Clearing-House	Encompasses all policy and regulatory frameworks (including instruments and activities) to manage risks associated with food and agriculture (including relevant environmental risks), including fisheries and forestry.
Biotechnology	Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for a specific use.
Capacity Building	Strengthening and/or development of human resources and institutional capacities.
Cartagena Protocol	The Cartagena Protocol on Biosafety to the Convention on Biological Diversity is an international agreement that aims

	to ensure the safe handling, transport and use of living modified organisms (LMOs) resulting from modern biotechnology, which may have adverse effects on biological diversity, also taking into account risks to human health. It was adopted on 29 January 2000 and entered into force on 11 September 2003.
Codex Alimentarius Commission	An international regulatory body (part of the FAO) responsible for the setting international food standards. The Commission periodically determines and publishes a list of food ingredients and maximum allowable levels (the Codex Alimentarius) deemed to be safe for human consumption.
Conference of the Parties	The Conference of the Parties to the Convention.
Consignment	A quantity of seeds, plants and plant products or any regulated article consigned from one country to other at any one time shipment and covered by a phytosanitary certificate, bill of entry of customs, shipping/airway bill or invoice.
Contained Use	Any operation, undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment.
Containment	Prevention of the spread of organisms outside the facilities, which may be achieved by physical containment (the use of good work practices, equipment and installation design) and /or biological containment (the use of organisms that have reduced ability to survive or reproduce in the environmenf).
Convention on Biological Diversity	The Convention on Biological Diversity (CBD) is an international treaty that was adopted at the Earth Summit in Rio de Janeiro in 1992 and entered into force on 29 December 1993. The Convention has three main goals, (i) conservation of biological diversity (ii) sustainable use of its components and (iii) fair and equitable sharing of benefits arising from use of genetic resources.

Event	Event is the insertion of a particulartrans gene into a specific location on a chromosome. Every cell that successfully incoronates the gene of interest represent a unique event.
Export	Intentional transboundary movement from one Party to another Party.
Exporter	Any legal or natural person, under the jurisdiction of the country of export, who arranges for a living modified organism to be exported.
Global Environment Facility	Launched in 1991, the Global Environment Facility (GEF) provides grant and concessional funds to the developing countries for projects and programmes targeting global environmental issues as climate change, biological diversity, international waters, ozone layer depletion, land degradation and persistent organic pollutants. Its implementing agencies are United Nations Environment Programme (UNEP), United Nations Development Programme (UNDP), and the International Bank for Reconstruction and Development (IBRD) commonly known as World Bank etc.
Gene	The fundamental physical and functional unit of heredity. A gene is typically a specific segment of a chromosome and encodes a specific functional product (such as a protein or RNA molecule).
Gene Expression	The process by which a gene produces mRNA and protein, and hence exerts its effect on the phenotype of an organism.
Genetic Engineering	The technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside an organism or the cell is inserted into the said organism/cell. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self-cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material (Rules, 1989).
Genetically	An organism that has been transformed by the insertion of

Modified Organism (GMO)/Genetically Engineered Organism (GEO)	one or more transgenes i.e. any living organism, the genes or genetic material of which has been modified in a way that does not occur naturally through mating or natural recombination or both.
Germplasm	Plants in whole or parts and their propagules including seeds, vegetative parts, tissue cultures, cell cultures, genes and DNA based sequences that are held in a repository or collected from wild as the case may be, and are utilised in genetic studies or plant breeding programmes for crop improvement.
Import	An act of bringing into any part or place of territory of the country any kind of seed, plant or plant product and other regulated articles from places outside India either by sea, land, air or across any customs frontier.
Importer	Any legal or natural person, under the jurisdiction of the country of import, who arranges for a living modified organism to be imported.
Import Permit	An official document that allows the import of any resource into the country from outside. An official document authorizing importation of a consignment in accordance with specified phytosanitary requirements.
Inspection Authority	Authority specified in Part I of Schedule XI of Plant Quarantine (Regulation of Import into India) Order 2003 or an Officer of the Directorate of Plant Protection, Quarantine & Storage duly authorized by the Plant Protection Adviser (PPA) for the purpose of approval and certification of post-entry quarantine facilities and inspection of growing plants in such facilities in accordance with guidelines issued by PPA or for any specified purpose, an authority specified in Part II of the said Schedule.
Interception (of a pest)	The detection of a pest during inspection or testing of an imported consignment (http://www.ippc.org).
Issuing Authority	Authority as envisaged under Schedule-IV of Plant Quarantine (Regulation of Import into India) Order 2003 or duly notified

	by the Central Government from time to time either generally or specifically for issuance of import permit.
Living Modified Organism	Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology.
Modern Biotechnology	The application of *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, fusion of cells beyond the taxonomic family that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.
Notification	A notification published in the official Gazette and the expression "notifies" shall be construed accordingly.
Pathway	Any means that allows the entry or spread of a pest (http://www.ippc.org).
Pest	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plant or plant products (http://www.ippc.org). It includes insects, mites, nematodes, fungi, bacteria, viruses, phytoplasma, rickettsia like organisms and weeds.
Phytosanitary Certificate	An official document confirming that the biological material received in the country from foreign sources is free from exotic pests and diseases. A certificate issued in the model format prescribed under the International Plant Protection Convention of the Food & Agriculture Organisation and issued by an authorized officer at the country of origin of consignment or re-export.
Plant Quarantine	All activities designed to prevent the introduction or spread of quarantine pests or to ensure their official control (http://www.ippc.org).

Plant Quarantine Order 2003	Plant Quarantine (Regulation of Import into India) Order, 2003 commonly referred as PQ Order, 2003 is the regulation for the purpose of prohibiting and regulating the import into India of agricultural articles and came into force on January 1, 2004.
Point of Entry	Any sea port, airport, or land-border check-post or rail station, river port, foreign post office, courier terminal, container freight station or inland container depot notified as specified in Schedule I or Schedule-II or Schedule-III of Plant Quarantine (Regulation of Import into India) Order, 2003.
Post-entry Quarantine	Growing of imported plants in confinement for a specified period of time in a glass house, screen house, poly house or any other facility, or isolated field or an off-shore island that is established in accordance with guidelines/standards and are duly approved and certified by an inspection authority notified under Plant Quarantine (Regulation of Import into India) Order, 2003.
Prior Informed Consent	Recognizes indigenous people's inherent and prior rights to their lands and resources and respects their legitimate authority to require that third parties enter into an equal and respectful relationship with them, based on the principle of informed consent.
Quarantine	Isolation for a period after arrival in a new location, to allow any pre-existing disease symptoms to appear. Used in the context of regulations restricting the sale or shipment of living organisms, usually to prevent disease or pest invasion of an area.
Quarantine Pest	A pest of potential economic importance to the area endangered thereby and present there but not widely distributed and being officially controlled (International Standards for Phytosanitary Measures- 5) (http://www.ippc.org).
Recombinant DNA	The result of combining DNA fragments from different sources.

Transboundary Movement	Movement from an area under the national jurisdiction of one state to or through an area under the national jurisdiction of another state or to or through an area not under the national jurisdiction of any state.
Transgene	The introduction of a gene sequence used to transform an organism. Often, but not always, the transgene has been derived from a different species than that of the recipient.
Transgenic Plants	Transgenic plants possess a gene or genes that have been transferred from a different species using recombinant DNA technology. The aim is to design plants with specific characteristics by artificial insertion of genes from other species or sometimes entirely different kingdoms.

ACRONYMS

AIA	Advance Informed Agreement
AOAC	Association of Analytical Chemists
BCH	Biosafety Clearing-House
BDA	Biological Diversity Act, 2002
BIRC	Biosafety Information Resource Centre
BRL	Biosafety Research Level
Bt	*Bacillus thuringiensis*
CAC	Codex Alimentarius Commission
CBD	Convention on Biological Diversity
CCC	Central Compliance Committee
CFTs	Confined Field Trials
CICR	Central Institute for Cotton Research
CNA	Competent National Authority
COP-MOP	Conference of the Parties -Meeting of the Parties
CPB	Cartagena Protocol on Biosafety
DAS-ELISA	Double Antibody Sandwich-Enzyme-linked Immunosorbent Assay
DBT	Department of Biotechnology
DLC	District Level Committee
DNA	Deoxyribonucleic Acid
DPPQS	Directorate of Plant Protection, Quarantine and Storage
DS	Dip Stick

EBAM	Event Based Approval Mechanism
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Environment (Protection) Act, 1986
ERA	Environmental Risk Assessment
ESTs	Event Selection Trials
FAO	Food and Agriculture Organization
GEAC	Genetic Engineering Appraisal Committee
GEO	Genetically Engineered Organism
GEF	Global Environment Facility
GIPSA	Grain Inspection, Packers and Stockyards Administration
GMO	Genetically Modified Organism
GURT	Genetic Use Restriction Technology
HT	Herbicide Tolerance
IBSC	Institutional Biosafety Committee
ICAR	Indian Council of Agricultural Research
ICGEB	International Centre for Genetic Engineering and Biotechnology
IGMORIS	Indian GMO Research Information System
IP	Import Permit
IPPC	International Plant Protection Convention
IR	Insect Resistance
ISAAA	International Service for the Acquisition of Agri-Biotech Applications
ISTA	International Seed Testing Association
JRC	Joint Research Centre
LAMP	Loop Mediated Isothermal Amplification

LFD	Lateral Flow Device
LFT	Lateral Flow Test
LMO	Living Modified Organism
LMO-FFPs	Living Modified Organisms for Food, Feed and Processing
LMO-UIds	Living Modified Organism-Unique Identifiers Registry
LOD	Limit of Detection
MoA&FW	Ministry of Agriculture and Farmers' Welfare
MoEF&CC	Ministry of Environment, Forest and Climate Change
NACEN	National Academy of Customs, Excise and Narcotics
NACIN	National Academy of Customs, Indirect Taxes & Narcotics
NBA	National Biodiversity authority
NBPGR	National Bureau of Plant Genetic Resources
NFP	National Focal Point
NOC	No Objection Certificate
OECD	Organisation for Economic Co-operation and Development
PCR	Polymerase Chain Reaction
PGR	Plant Genetic Resources
PEQI	Post-entry Quarantine Inspection
PQ	Plant Quarantine
PSC	Phytosanitary Certificate
RCGM	Review Committee on Genetic Manipulation
RDAC	Recombinant DNA Advisory Committee
RT-PCR	Reverse Transcription-Polymerase Chain Reaction

SAU	State Agricultural University
SBCC	State Biotechnology Coordination Committee
SOPs	Standard Operating Procedures
SPS	Sanitary and Phytosanitary
TC	Tissue Culture
UNDP	United Nations Development Programme
UNEP	United Nations Environment Programme
VP	Vegetative Propagules

CONTRIBUTORS

Ahuja Vibha	Chief General Manager Biotech Consortium India Limited Anuvrat Bhawan Deen Dayal Upadhayay Marg, New Delhi 110002
Bhalla Shashi	Emeritus Scientist (Former Principal Scientist) Division of Plant Quarantine ICAR-NBPGR, New Delhi 110002
Chimata Murali Krishna	Joint Director/ Scientist-D MoEF&CC, Govt. of India Indira Paryavaran Bhawan, Jor Bagh, New Delhi 110002
Dubey SC	Principal Scientist and Head Division of Plant Quarantine ICAR-NBPGR, New Delhi 110002
Maurya AK	Chief Technical Officer Division of Plant Quarantine ICAR-NBPGR, New Delhi 110002
Priyadarshini Chanda	Project Associate Division of Plant Quarantine ICAR-NBPGR, New Delhi 110002
Randhawa Gurinderjit	Principal Scientist and Officer-In-charge Division of Genomic Resources ICAR-NBPGR, New Delhi 110002
Sharma Ruchi	Senior Research Fellow Division of Plant Quarantine ICAR-NBPGR, New Delhi 110002
Singh Kuldeep	Director ICAR-NBPGR, New Delhi 110002
Singh Monika	Scientist Division of Genomic Resources ICAR-NBPGR, New Delhi 110002
Tripathi Aradhika	Research Associate Division of Plant Quarantine ICAR-NBPGR, New Delhi 110002
Vasimalla Celia Chalam	Principal Scientist Division of Plant Quarantine ICAR-NBPGR, New Delhi 110002

ISBN 978-81-937111-2-5