

GPG2, Activity 2.1.2: “Develop crop-specific guidelines to maintain germplasm free from transgenes”

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Participants and contributors	2
Output of activity 2.1.2	2
Review of the “Guiding principles for the development of Future Harvest Centers’ policies to address the possibility of unintentional presence of transgenes in <i>ex situ</i> collections”	2
Development of specific guidelines to maintain germplasm free from transgenes	2
Testing to detect unintentional transgenes	3
Sample size	3
How to test	3
When to test	3
Positive results and their publication	3
If a sample tests positive: Crisis management responses	4
GMO free declaration	4
References	4
Table 1. Schematic description of the guidelines for the detection of the unintentional presence of transgenes in genebank Accessions	6
Figure 1. Sequence of procedures in a genebank	10

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Output of activity 2.1.2

One document with:

- Review of the “Guiding principles for the development of CGIAR Centers policies to address the possibility of unintentional presence of transgens in ex-situ collections” used in guideline development.
- Conceptual framework: based on the guiding principles, and table 1
- Three crop-specific chapters, based on the conceptual framework (not necessarily in the same order or format, but containing all the required elements)
- General recommendations, such as that establishment of crop-specific regulations must be in accordance with national and institutional biosafety laws and regulations
- Recommendations for funding (may include cost estimates and outsourcing options)
- External links (both crop-specific & general), including to OECD biosafety and risk assessment, ISTA, FAO

Desirable appendices:

- Activity follow-up, including information on sustainability management
- FAQ
- Crisis management responses
- Future projections with regards to this subject

Review of the “Guiding principles for the development of CGIAR Centers’ policies to address the possibility of unintentional presence of transgenes in *ex situ* collections”.

Among the 15 sections that form the “Guiding principles”, the following points were identified for development into crop-specific guidelines, as required by this project:

8. Evaluation of genebank operations in order to identify the possible risks of gene flow between the bank materials and transgenic events present in the environment
9. When to test new acquisitions
10. How to test for the unintentional presence of transgenic events in existing accessions
11. How to deal with the detection of an unintentional transgene presence
12. Maintenance of a database on a publicly available website
15. How to deal with the publication of test results and the announcement of a positive interception

Development of specific guidelines to maintain germplasm free from transgenes

Table 1 gives a schematic description of the guidelines. The crop-specific guidelines for maize, potato and rice, prepared by CIMMYT, CIP and IRRI respectively in accordance with this structure, follow in the subsequent chapters.

The basic operations occurring in a genebank, from the acquisition of a new accession to final storage and distribution, are defined in figure 1. It is understood that for specific crops these operations may differ slightly.

The risk under discussion is defined as the occurrence of an unintentional introduction of a transgene or transgenes into a genebank collection during each operation.

Indoor/internal (contamination, mixture) and outdoor/external (other released germplasm, experimental germplasm) sources of this risk associated with each operation have been identified and described.

The risk management measures are described in table 1 at a non-crop-specific level and in the following chapters at a crop-specific level.

Laboratory testing for GMO detection is recommended as a management measure, depending on the bank operation involved and on the identification and characterization of the source(s) of risk.

Different levels of testing procedures are suggested according to three levels of risk:

- High:
 - refusing introduction of an accession
 - testing all germplasm to achieve the highest level of confidence
 - testing using small initial sample size
- Medium: testing using partial or probabilistic sampling methods to achieve a situation-specific result (depending on crop, source, etc.) at a defined level of confidence
- Low: no testing, because either no transgenic events are present in the area where the sample was grown or there is no source of risk of gene flow

Other risk management measures that do not involve testing are described for each operation.

Testing to detect unintentional transgenes

Sample size

The decision on the sample size to test is guided by:

- statistical indications that can be found in Hernandez-Suarez et al. (2008) and other sources (e.g., GIPSA, ISTA; see website references)
- the amount of seed available
- the testing procedure used

How to test

The decision regarding how to test the material is crop specific, but it is agreed that DNA-based (rather than protein-based) detection will be essential. It is predicted that in the future, all approved transgenes will have publicly and commercially available markers and detection procedures. Therefore, it is necessary to retrieve and maintain updated information on transgenic constructs and respective detection methods. A website with links to the most accredited external database(s) will be prepared. Nevertheless, some of this construct information may be kept confidential and hence we may not have access to it.

When to test

Testing should potentially be carried out at two key stages of bank operations:

- On new acquisitions, including *in situ* collections and material originating *ex situ*, at the point of entry into a genebank
- On existing accessions before initial use, regeneration, and distribution

Positive results and their publication

The participants recommend adopting a cautious approach, respecting the moral integrity of our partners in providing genetic material in good faith, while also recommending full transparency where the provider is aware of the presence of transgenes in the material provided.

Where a positive result is obtained, confidentiality and consultation with the provider in the first instance must be respected in order to obtain all necessary information regarding the material. The participants felt that genebank managers should follow the same principles as for the unintentional presence of pathogens and not become a “global GM detection police”. Material

found positive should usually be destroyed and the provider informed. If the material is accepted by the genebank, the center also has a moral obligation to inform the national biosafety authority.

If a sample tests positive: Crisis management responses

- For a new acquisition the following possibilities are suggested:
 - Retest to verify
 - Destroy or return to the provider
 - Maintain the sample noting the possible presence of the transgene(s)
 - Scrutinize possible sources of contamination
 - Enter into a confidential dialogue with the provider (or donating country authority)
 - If the materials are accepted into the genebank, national and institutional regulations must be followed, with public (transparent) release of all informationPublic announcement of the findings will be made at the discretion of the provider (except where the material enters the genebank).

- For an existing accession the following possibilities are suggested:
 - Retest to verify
 - Identify the gene constructs
 - Determine level of presence of the transgenes
 - Inform past recipients, if the accession has previously been distributed
 - Inform the provider (without accusing)
 - Inform other genebank holders of the accession (identified through crop registries)
 - Maintenance of the sample will depend on individual bank: as is, or “cleaned” if deemed necessary. Follow guidelines for recognized transgenic accessions
 - Try to minimize any institutional damage

- For an existing accession that has been distributed and is in a commercialized form the following suggestions are made:
 - Formation of a crisis-management team (including institutional management, legal advisor, communications expert, biosafety expert, scientist(s))
 - Use of a crisis management reaction matrix (a tool for PR management) to produce a communication strategy
 - Prompt (proactive) timing is essential
 - Issue a global public alert.
 - High transparency will result in fewer negative consequences than secrecy. Transparency should also be maintained during ordinary times by cultivating public media that are informed on the types and aims of GM research at CGIAR centers, as an “educational” activity that may help ameliorate the impact when an undesirable result must be made public

GMO free declaration

GMO “free” statements can not be issued for technical reasons, except for clonally propagated crops (at high costs). However, transparency is essential, and may require clear statement of procedures used to ensure that materials have a low probability of containing transgenes. Declarations can be issued within stated thresholds and with stipulations such as “to the best of our knowledge”.

References

Hernandez-Suarez, C.M, O. Montesinos-Lopez, J. Crossa, and G. McLaren. 2008. Probability models for detecting transgenic plants.. *Seed research Science* 18: 77–89.

The following websites provide descriptions of transgenic events and databases on the events released for each crop in each country; the international biosafety agreement; and technical information on sampling and testing.

AGBIOS <http://www.agbios.com/dbase.php?action=ShowForm>

Convention on Biological Diversity <http://www.cbd.int/convention/default.shtml>

Cartagena Protocol on Biosafety <http://www.cbd.int/biosafety/protocol.shtml>

Grain Inspection, Packers and Stockyards Administration (GIPSA)

Homepage:

<http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=landing&topic=landing>

Sampling procedures:

<http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=grpi&topic=rd-bi>

GMO ERA Project <http://www.gmo-guidelines.info/index.html>

International Service for the Acquisition of Agri-biotech Applications (ISAAA)

<http://www.isaaa.org/inbrief/default.html>

International Seed Testing Association (ISTA) <http://www.seedtest.org/en/home.html>

Sistema de Información de Organismos Vivos Modificados

http://www.conabio.gob.mx/conocimiento/bioseguiridad/doctos/consulta_SIOVM.html

OECD BioTrack Product Database <http://www2.oecd.org/biotech/>

Table 1. Schematic description of the guidelines for the detection of the unintentional presence of transgenes in genebank accessions.

Operation	Definition and description of activity	Risk factors	Risk management according to level of risk
Collection	In-country contributions. Obtaining germplasm from collecting missions (farmer's fields, markets) or <i>in situ</i> wild habitats.	<ol style="list-style-type: none"> 1. Existence and level of enforcement of country biosafety regulations 2. Existence of GM technologies, GM germplasm and varieties in crop or related species (globally, in-country and regionally) 3. If GM varieties exist, their prevalence in the country and region of collection 4. Markets in-country or regionally where GM varieties are available 5. Presence of GM in-country or regionally in grain, food, or seed provided as aid 6. Distance to the nearest GM field 7. Presence of "bridging species" (volunteer and feral GM plants) and viable descendents of past GM crops 8. Evidence of geneflow occurrence 9. Possibility of escape from nearby research and development facilities, field testing sites and biosafety facilities 10. Proximity to ports of entry or transportation arteries 11. Degree of informal seed exchange ("pocket breeding", among farmers) 12. Relevance of existing GM varieties to farmers 13. Availability of adequate screening methodologies 	<p><u>If low:</u> no testing</p> <p><u>If medium:</u> testing to the predetermined level of probability</p> <p><u>If high:</u> a. no collection b. testing of all samples collected, with the option of reducing the sample size</p> <p>If test result is positive: see section "Testing"</p>

Acquisition/ Introduction	Obtaining germplasm from <i>ex situ</i> sources (centers, genebanks, individuals scientists, private growers, seed companies, etc)	<ol style="list-style-type: none"> 1. Ability of the provider to assess, manage and document the unintentional presence of transgenes 2. Availability of information from the provider on germplasm origin and testing for transgenes 3. Availability of adequate screening methodologies 	As above except for action in case of positives: Append germplasm Acquisition agreements, or Need to draft an imported seed production declaration.
Regeneration	<ol style="list-style-type: none"> a. Multiplication in the field or in a confined environment (greenhouse, screenhouse, laboratory) b. Delivery back to genebank 	<ol style="list-style-type: none"> 1. Mixtures due to improper isolation from GM sources 2. Inadequate isolation of perennial field collections 3. Volunteer plants 4. Vandalism 	<ol style="list-style-type: none"> 1. Application of “best practices”: preventive measures in the field or confined environment to avoid gene flow, volunteers and mixtures into the collection (including borders, barriers, pollination practices, and monitoring of experiment stations for unintentional GM presence) 2. Proper practices during delivery to the genebank (packing and transportation) 3. Maintenance of regeneration history 4. Maintenance of GMO identity if necessary
Characterization	Observation and registration of highly heritable physical, morphological and molecular characteristics	<ol style="list-style-type: none"> 1. If characterization occurs during regeneration and the harvested propagule is returned to the bank, as for regeneration 2. If separate experimentation is carried out and the harvested propagule is returned to the bank, as for regeneration 3. For laboratory characterization, as for other laboratory procedures 	As for regeneration

Conservation	<ul style="list-style-type: none"> a. Seed: physical cleaning, sorting, drying, packaging and storing b. Vegetative: cryo-conservation, in-vitro, field 	<ul style="list-style-type: none"> 1. Mixtures due to lack of physical separation during harvest and cleaning 2. Existing materials that have been conserved and regenerated without recognizing the possible presence of transgenes (i.e. without recognizing source) 3. Volunteer plants 4. Vandalism 5. Materials collected and introduced after the occurrence of the first transformation without following the procedures described in these guidelines 6. Materials collected before the occurrence of the first transformation but regenerated afterwards 7. Improper isolation from GM sources during conservation of <i>ex situ</i> field collections 	<ul style="list-style-type: none"> 1. Laboratory testing to provide quality assurance (including periodic blind testing) 2. Preventive measures to avoid gene flow, volunteers and mixtures into the collection 3. Use (distribution, regeneration, scientific purposes) of backup materials subjected to the procedures described in these guidelines before utilization
Testing (health, viability, GM)	<ul style="list-style-type: none"> a. Detection of relevant pathogens, pests, and parasitic and noxious weeds b. Testing of the extent to which a propagule is alive (germination, tetrazolium and other laboratory testing) c. Testing of the extent to which a propagule can establish a plant 	<p>If the material tested re-enters the bank:</p> <ul style="list-style-type: none"> 1. Mixtures due to lack of physical separation 2. Level of quality standards for testing and procedures (errors in labeling samples etc) <p>Destructive testing reduces risk.</p>	<ul style="list-style-type: none"> 1. Separation in time or space of GM and non-GM research facilities 2. Control and increase of quality standards In laboratory testing procedures
Curative treatment	Application of chemical, biological, and physical methods to eliminate pathogens and pests	As for testing Accuracy in maintenance and cleaning of the equipment used for treatment	As for testing
Evaluation	Assessment of performance	As for characterization	
Distribution	Extracting germplasm from the bank, re-packaging if required, shipment, and compliance with institutional, national and international requirements (phytosanitary, biosafety/GMO, customs, export, IP; WTO, FAO, Cartagena, ITPGRFA)	<ul style="list-style-type: none"> 1. If re-packing is necessary and the residual material re-enters the bank: <ul style="list-style-type: none"> a. Mislabeling b. Mixtures 2. Undetected transgenes due to errors in the sampling procedures adopted for testing before conservation 3. Inadequate collation of up-to-date information 	<ul style="list-style-type: none"> 1. Quality management of bank procedures 2. Updated information for compliance

Documentation	The organized collection of records that describe genebank structure, purpose, operation, maintenance, and data requirements. It includes: <ul style="list-style-type: none"> a. Acquisition documentation (including MTA, IP) b. Accession maintenance documentation (including initial sample size) c. GMO status (including identification of specific constructs, if present) d. Data sharing, including confidential data management, relevant public data and organization in forms visible to others (field book, database, web site, 	Registration of wrong information	Quality management of bank procedures

Figure 1. Sequence of procedures in a genebank

