Practices and procedures to avoid the unintentional presence of transgenes in maize germplasm accessions at the Wellhausen-Anderson Plant Genetic Resources Center

Contents

SUMMARY

This document sets out proposed practices to avoid the possibility of the unintentional presence of transgenes in CIMMYT's maize collection, in particular to:

- minimize, as far as possible, the probability of unintentionally introducing transgenes into the collection;
- establish an effective program of testing for the presence of transgenes; and
- establish an effective response strategy to deal with the case that a transgene is discovered in supposedly non-transgenic maize.

The strategy to be followed by CIMMYT is to adopt those practices that most effectively minimize the probability of unintentionally introducing transgenes, and to identify critical risks of their introduction during collection and acquisition of material, so that we minimize the need for unnecessary testing for the presence of transgenes.

The document has been developed following the "Guiding principles for the development of CGIAR Centres' policies to address the possibility of unintentional presence of transgenes in *ex situ* collections", published and adopted by all CGIAR centres in 2005 following an Expert Workshop on "technical issues associated with the development of CGIAR policies to address the possibility of adventitious presence of transgenes in CGIAR *ex situ* collections".

BACKGROUND: THE GENETIC STRUCTURE OF AN ACCESSION AND ITS MAINTENANCE

1. In 1998 Mexico banned the import and growth of transgenic maize. This should guarantee that the maize germplasm collection is not exposed to transgenes during regeneration and characterization on CIMMYT research stations or through collection of new accessions in Mexico. On the other hand, CIMMYT receives several hundred new accessions each year from other countries, which could have been exposed to risks of transgene flow in their countries of origin.

2. In 2005 Mexico implemented a biosafety law that regulates the import, production and use of transgenic crops, including maize. In March 2009 the Mexican government issueed a regulation that allows the planting of transgenic maize in open fields; to date no permissions have so far been granted.

3. In the management of its maize germplasm collection, CIMMYT employs the following principles: equity in seed distribution, documentation for the passport, characterization, evaluation, utilization, seed viability and purity quality. CIMMYT operates within the framework of the multilateral system of germplasm exchange and use under the International Treaty for Plant Genetic Resources.

CIMMYT's maize bank collects new and unique diversity, conserves it, and makes it available to the international user community. The maintenance of the genetic structure of each accession in the bank during seed processing, regeneration and increase may be threatened by the unintentional introduction (through contamination or introgression) of foreign genes from other accessions or other sources, including transgenes. CIMMYT therefore takes proactive steps that aim to prevent any change in the genetic structure of accessions by genes not present in the original samples, by following proper genebank management procedures and practices. It monitors new and regenerated accessions by employing protocols for the detection of unintentional genes.

4. Transgenes and conventional genes are subject to the same underlying biological processes of selection, mutation, and recombination. Therefore, best practices for preventing the unintentional introduction of conventional genes provide an appropriate basis for preventing the introduction of transgenes.

5. During seed regeneration or increase in the field, CIMMYT uses artificially-controlled hand-pollination to prevent the introduction of genes from sources other than the accession in question. Shoot bagging before the emergence of the silks and tassel bagging to capture pollen grains one day before hand pollination are performed for each plant. Roguing of off-types within the plot is carried out before the

pollination.

6. Despite the adoption of proper field operations, a possibility remains of the introduction of foreign or unwanted genes, including transgenes, to the accessions. Available testing techniques do not provide an absolute guarantee without testing every single seed or plant. However, best practices in bank operations will achieve a high degree of statistical probability that the accession does not include unintentionally-introduced transgenes.

7. Factors that can contribute to the risk of the introduction of transgenes to bank accessions are assessed for each stage of genebank operations: collection, acquisition, regeneration, distribution, health testing, viability testing, preservation, characterization, evaluation, documentation, and data sharing.

COLLECTION

Risk assessment

In situ collection on farm and in wild habitats (for teosinte) represents the greatest source of risk of the unintentional introduction of transgenes.

Maize is an outbreeding crop. Open-pollinated varieties may receive pollen from other maize varieties or hybrids, including transgenic hybrids. Thus the risk of transgenes appearing in conventionally-bred varieties through natural introgression from transgenic varieties is higher than for inbreeding crops. Landraces can also be exposed to transgene flow if these are present in the vicinity.

In Mexico there have been reports of transgene flow into maize landraces from illegal introduction of transgenic maize seed or use of transgenic maize grain imported for feed and food. Therefore, the potential risk from these transgene sources should be taken into account at the time of collection on-farm. In case of collection in countries other than Mexico, it is necessary to retrieve all possible information on the events released and on governmental regulations regarding the use of transgenic crops.

The general risk of transgenes appearing in non-transgenic maize varieties in a particular country or region depends on a number of factors:

- The existence and enforcement of governmental regulations on transgenic maize in the country
- The existence and implementation of procedures to monitor the presence of transgenes in the country
- The adoption of transgenic technologies: the permitted growth of transgenic hybrids in the country with public acceptance and marketing transparency
- The presence in markets or in aid shipments of imported transgenic maize grain, possibly not labelled as transgenic, or of imported grain of conventional hybrids or varieties from high-risk countries
- The degree of informal seed exchange among farmers in high-risk areas

Within a country or region, the specific risk at a site of collection also depends on other factors, such as:

- Proximity to research and development facilities and field testing sites where transgenic maize is knowingly studied

- Proximity to research and development facilities and field testing sites that frequently introduce new germplasm for testing, and are therefore at higher risk of unknowingly introducing transgenic maize

- Proximity to ports of entry, processing factories or transportation arteries, where volunteer and feral maize plants may be relatively abundant

- Distance to the nearest transgenic field

Risk management

Before any new collection mission is undertaken, a specific review of the level of risk must be carried out for each of the above risk factors.

If the risk is judged to be high or medium, whether across the whole region or in certain locations, project funding for the collection mission should include the cost of testing some or all of the collected samples. If such funding is not forthcoming, samples should not be collected from locations where the risk is medium or high. If the whole region covered by the mission is considered medium to high risk, the collection mission should be aborted, particularly for on-farm locations, to avoid a more complicated task of screening the samples for transgenes at a later stage.

If there are known sources of transgenic maize in the collection region, the passport data of the samples should record the possible sources of transgenes at collection and the specific collection sites with a relatively high risk of the unintentional presence of transgenes.

Monitoring and containment

If there is a risk of the unintentional presence of transgenes in the samples collected they should be handled separately and isolated until they are known not to be transgenic.

All samples collected where the risk of the presence of transgenes is real or expected, regardless of the level of contamination (high, medium or low), should be tested according to the following procedure:

- Sample the appropriate quantity of well-mixed seed according to the size of the sample collected and determine the method that will be used for analysis

- Put aside the remaining seed for preservation, seed increase or regeneration in an isolated area. This sample should not be used until the results of the analysis are available

- If no transgene is detected, the genebank procedures and practices used for non-transgenic seed accessions can be followed to add the new accessions to the maize collection

- If one or more transgenes are detected, measures should be taken following the center's policy for the management of transgenic accessions and the Mexican authorities should be immediately informed of the interception

ACQUISITION

Risk assessment

As for *in situ* collection on farm, acquisition or introduction of germplasm from an *ex situ* source (e.g., another bank, a breeder, university, research institute, etc) also represents a significant source of risk, as information associated with the sample regarding its exposure to transgenes may be wrong or lacking.

In the *ex situ* acquisition of samples, the recipient should know the origin of the germplasm (provided in the sample's passport); the standards and procedures followed by the provider to maintain the genetic integrity of the sample and monitor transgene presence; and the steps taken to ensure compliance with relevant local biosafety regulations.

Several factors contribute to the risk of introducing transgenes through *ex situ* acquisition of germplasm, including:

- The presence of transgenes in the region where the provider obtained the sample

- The presence of transgenes in the area where the provider planted the sample, including any of the provider's own research and breeding with transgenic maize

- The provider's compliance with good germplasm management practices according to institutional, local and national biosafety standards

- The ability of the provider to assess or screen, manage, and document the unintentional presence of transgenes

Risk management

Before any maize is acquired from an *ex situ* source, the potential provider should be asked to provide information on the transgenic status of the sample. To ensure that sufficient information is obtained to make a sound judgment on the level of risk, the provider should be asked to complete the following questionnaire:

1. Is transgenic maize grown in the country of origin of the sample?
2. Does your organization handle transgenic maize?
3. What other potential sources of maize transgenes might there be, known or unknown, in the region where you work?
4. What official local or national biosafety regulations are in force in the country of origin of the sample?
5. What institutional, local and national biosafety standards do you follow to keep transgenic maize separate from non-transgenic maize and to prevent the unintentional presence of transgenes in the sample?
6. Do you follow documented best practices for germplasm management? If so, which ones?
7. How far was the sample from the closest transgenic maize field?
8. Was the sample tested before shipping?
9. If you have tested the sample and failed to detect transgenes,
    a. What procedure did you use to detect transgenes?
    b. What minimum frequency of transgenes can your test procedure detect, with what confidence?

If you have not tested the sample, please answer the following questions:

10. Did you breed the sample yourself?
11. If you did not breed the sample,
    a. When did you obtain it?
    b. From where did you obtain it?
    c. Did you acquire it with any statement or analysis of the presence of transgenes at the time of acquisition?
    d. What do you know about the likelihood of the intentional or unintentional presence of transgenic maize in the region of the provider at the time of acquisition?

On the basis of the information received by the provider the detection limit for transgene testing must be decided to be able to properly perform the acquisition process and appropriate testing for the presence of transgenes in the sample.

Monitoring and containment

CIMMYT must follow host country phytosanitary and biosafety regulations in importing maize germplasm from other countries. Every sample accepted, regardless of the level of risk, should be tested according to the same procedure outlined above for collection. In the event of the discovery of transgenes in a sample, the provider should be informed and the sample destroyed, returned or isolated until its final destination is determined. CIMMYT germplasm bank policy follows CGIAR guidelines on procedures to avoid the unintentional introduction of transgenes.

REGENERATION

Risk assessment

In common with other annual outbreeding crops, the maize accessions are normally planted in the field plots for regeneration. The regeneration of accessions is performed by the bank for rejuvenation and seed multiplication purposes. Artificial hand-pollination is carried out during regeneration using shoot bags and pollen-protector bags. Some genetic change during regeneration is inevitable in the case of panmictic populations of the accession because of the sample size used and the pollination procedure employed by the bank manager. Like any gene, transgenes may be incorporated into an accession during regeneration if:

- Foreign pollen migrates from neighboring plots, or from nearby plots or fields outside the control of the bank, to the regeneration field
- There is inadequate isolation from sources of transgenic pollen during anthesis
- Incorrect pollinations are performed with plants of different plots or volunteer plants in the same plot
- There are failings in the field and bank operations in the regeneration process, in any of the steps of seed preparation, sowing, pollination, harvest, seed processing or seed deposit in the bank
- There are inadequate safeguards against human factors such as vandalism and mislabeling

As the development and use of transgenic maize expand, and especially if field releases of transgenic maize in Mexico are approved, the risk of pollen contamination in the regeneration fields will increase. In order to monitor and prevent the possibility of uncontrolled migration or introduction of transgenic events on its research stations, CIMMYT has held an internal policy since 2003.

Risk management

The following risk management procedures are in place as an essential part of standard germplasm regeneration procedures to prevent contamination from foreign pollen or admixture with foreign seed:

- CIMMYT biosafety policy does not allow the planting of commercial maize hybrids on stations
- “Sentinel plots” to detect GM pollen are planted within CIMMYT research stations, taking into consideration the nearest potential pollen sources coming from outside the research station, as well as other breeding materials, and the prevailing wind direction. A blend of hybrid seeds or varieties representing a wide range of flowering dates are used in order to collect sample pollen over the entire growing season of the regeneration plots; this may require more than one planting date for each sentinel plot.
- “Sentinel border rows” are also used for specifically monitoring the regeneration plots. Five rows of maize are planted at the plot edges and de-tasseled to

prevent contamination. These are used to monitor transgenes coming from neighboring plots or from outside the experimental station.

- CIMMYT undertakes full training or constant supervision of field workers to ensure they comply with the required standards for seed processing and pollination operations.

Monitoring and containment

At present, since no transgenic maize is commercially available in Mexico, the implementation of the above management procedures is considered sufficient to reduce the risk of transgenes being introduced during regeneration to a low level where monitoring can be carried out using protein-based detection techniques. If any transgene is detected in the monitoring or sentinel rows, all entries from the regeneration or breeding plot must be tested to remove the affected lines or accessions. A repeated planting of these lines would then be required to ensure the production of non-transgenic germplasm.

The same monitoring procedure could also be implemented in all experimental plots of breeding lines if the commercial growth of transgenic maize in Mexico becomes a reality, increasing the risks of contamination. Half-sibs or top-crossed progeny without artificial pollination are more vulnerable to contamination if sources of transgenic pollen are present. However, artificial pollination is a good practice that should prevent contamination of the silks by foreign pollen, ensuring that they receive pollen only from directed sources in breeding and regeneration operations.

SEED DISTRIBUTION

Risk assessment

Most of the maize accessions currently preserved in CIMMYT's genebank originated before 1997. The risk of the unintentional presence of transgenes in the original collection should be very small. Recently-collected samples are screened for transgenes before being introduced to the maize germplasm collection, either within CIMMYT using protein-based detection techniques, or using molecular analyses, which are outsourced. Therefore the risk of the unintentional presence of transgenes in the current CIMMYT maize germplasm collection is minimal to the best of our knowledge. Measures to minimize this risk are taken at all stages from the origin of the accession to seed regeneration and increase.

Risk management

Good laboratory practices, in addition to those adopted in field operations, must be followed during the preparation of seed for distribution, to ensure that seed packets are not mislabeled and that there is no mixture of seeds from different sources, for example through inadequate standards of personal attention or cleaning of seed counter equipment between successive samples. The sample preparation staff and helpers must either be fully trained or constantly supervised to ensure good laboratory practice.

Reducing the likelihood of the unintentional presence of transgenes in the bank automatically minimizes the risk of unintentionally distributing transgenes to others.

Monitoring and containment

No additional transgene detection testing is carried out before seed shipment, the procedures described above having been properly followed.

If a requestor asks for the material to be certified Genetically Modified Organism-free, the following responses will be offered:

- If the material has not been tested, the following statement will be made:
  "To the best of our knowledge the sample is free from genetically modified organisms. It was developed and produced solely by conventional methods without the use of genetic transformation technologies, and in isolation from any known source of potential contamination by genetically modified varieties. It has been managed in accordance with a series of protocols to minimize the likelihood of the unintentional presence of transgenes."

- If the material has been tested, the following statement will be made:
  "The seed has been sampled from material that has been tested for the presence of transgene(s) according to the procedure described in … within the limits of detection by the test, the sample was free of the said transgenes"

If a requestor asks for tests to be conducted, this will be offered at the requestor's expense. Two levels of testing may be provided:

- Testing one subsample to the requestor's required degree of certainty, and sending a separate subsample

- Using a subsample to regenerate a new generation, and testing every parental plant in that subsample before carrying out artificial pollination

DOCUMENTATION AND DATA SHARING

Risk assessment

Inaccurate documentation may lead to incorrect decisions on handling accessions.

Risk management

The priority in managing this risk is to ensure accurate documentation. Procedures and protocols for improving data accuracy on both seed and seed health, including transgene detection, are currently in place at CIMMYT. CIMMYT's practices are both institute-wide and internationally-recognized (with ISO accreditation of the Seed Health Laboratory) and within the context of the GPG2. The process of recording and encoding data is double-checked.

OTHER BANK OPERATIONS

Risk assessment

Other bank operations (including seed health testing and seed viability testing) are treated together here, since the factors contributing to the risk of the unintentional introduction of transgenes are essentially the same; all involve handling seed and the associated data.

Contributing factors include:

- Mislabeling of seed packets
- Mixing of seeds from different sources, for example through inadequate standards in handling the seed samples and equipment between successive samples
- Inadequate quality control, documentation of procedures, or training and supervision of staff

The same bank operations must also be carried out in the management of known transgenic maize, generating two distinct categories of risk:

- The risk of mislabeling or admixture from known transgenic maize
- The risk of mislabeling or admixture involving undetected transgenes in a supposedly non-transgenic genebank accession

Risk management

If transgenic maize is allowed in Mexico, CIMMYT will need to adapt its germplasm-management operations, implementing the following:

- The management of transgenic maize should take place separately from that of bank accessions, in different laboratories and other buildings
- Staff and administration should be independent, to ensure that transgenic material is not accidentally transferred by staff moving between transgenic and bank facilities
- Transport routes (for example between laboratory and field, or between laboratory and screenhouse) should be independent and non-overlapping
- Control plants, and any other plants harvested in any containment facility in which transgenic plants have ever been grown, should be managed like transgenic maize, i.e., they should be managed entirely separately from bank accessions
- Transgenic facilities (including cold room, drying room, seed handling room, transgenic greenhouses with high-level containment, analytical lab, and transformation lab) should be organized as far as possible into a single unit with controlled access and the highest level of containment, and with no external transport routes between facilities
- The movement of transgenic maize from place to place in viable form should be curtailed as much as possible, by:
    - incorporating seed-processing head-houses into low-level containment screenhouses
    - polishing grain (removing the embryo) before it leaves the bank for transfer to the adjacent analytical lab

Good laboratory practice in bank operations, already undertaken to prevent mislabeling and admixture, is equally essential to prevent the spread of undetected transgenes within the bank:

- All processes and workflows must be fully documented and implemented
- All equipment must be kept scrupulously clean, and in particular all remnant seed must be removed and disposed of after completing each process
- The correspondence between seed packet labels and data recording sheets should always be double-checked

Monitoring and containment

CIMMYT maize germplasm managers have long carried out best practices to conserve the genetic integrity of accessions and maintain their purity from foreign genes, including unintentionally-introduced transgenes. These include accurate and controlled documentation, field operations, seed handling operations, seed health and viability testing, seed shipment, and monitoring of new introductions. The best practices considered here should be performed now and in the future to maintain the quality of seed and information for the users of CIMMYT's maize accessions.